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# CEREAL CHEMISTRY

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No. 1

## MICRO-BAKING TECHNIQUE, APPLICATIONS AND RESULTS

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(Read at the Annual Meeting, May 1938)

Micro baking is a form of baking procedure on which relatively little work has been reported. Only a few references are to be found in publications of recent years. Werner (1932) did some unpublished work and his terse summation was that "the micro equals the pup divided by four." Geddes and Sibbitt (1933) did work on 25 and 50 g. formulas, described by themselves as limited, and concluded that flours can be satisfactorily differentiated in regard to loaf volume and other characteristics by small baking tests. Geddes and Aitken (1935) did further work in connection with an experimental mill of miniature size and reported a striking comparison in volume and other external characteristics but indicated difficulty in scoring internal factors. Van Scoyk (1937) described a miniature molding device which greatly facilitated handling small doughs and gave much better replication of results. Harris and Sanderson (1938) reported a comparison of 100-g. and 25-g. formulas, finding a significant correlation between the two methods as to volume, stating however that the cut surface of the loaf was too small to score for grain and texture.

### Apparatus and Technique

A single micro dough contains 25 g. of flour and the basic A.A.C.C. formula is used, with applications of such supplements as may be required for specific purposes. In fact, reduction of the flour quantity from 100 g. to 25 g. may be considered another supplement to the standard test. The apparatus used is pictured in Figures 1, 2, 3, and 4. Figure 1 shows comparative sizes of the 25-g. and 100-g. pans. Both pans are made of aluminum with the 25-g. pan proportionally scaled down to one-fourth the volume of the larger pan. The ordinary 8-ounce covered jelly glasses shown at the right are used for fermentation jars. Figure 2 shows a side view of the micro-molding assembly with

sheeting rolls on the left and compression drum on the right. Figure 3 shows an oblique top view of the molder. The doughs are given conventional punches by hand and molded by passing through the

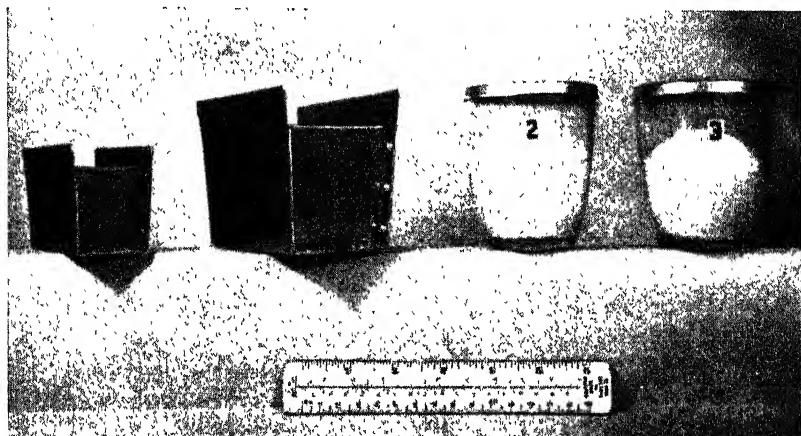


Fig. 1. Pan size comparison and micro fermentation jars.

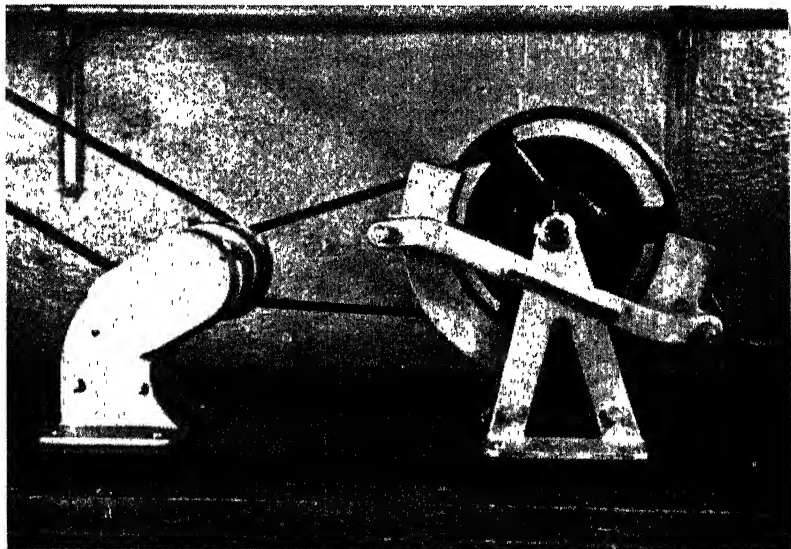


Fig. 2. Side view of micro molder.

sheeting rolls, curling loosely by hand, and then passing through the compression chamber. As to size, this molder is mounted on a base 8 inches by 25 inches and the compression drum is 8 inches in diameter. Standard proof is given the panned dough and baking is carried out

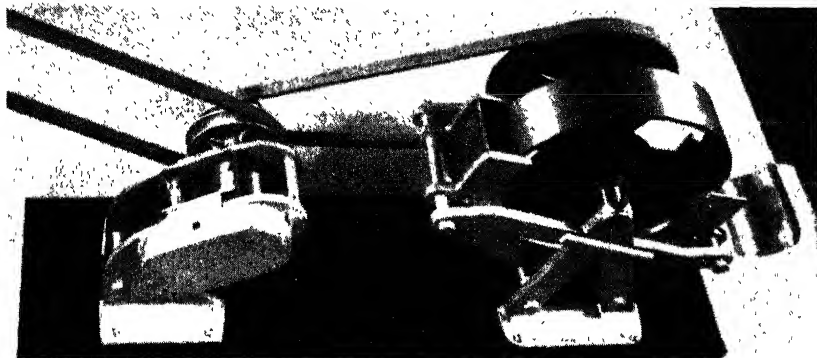


Fig. 3. Top view of micro mold.

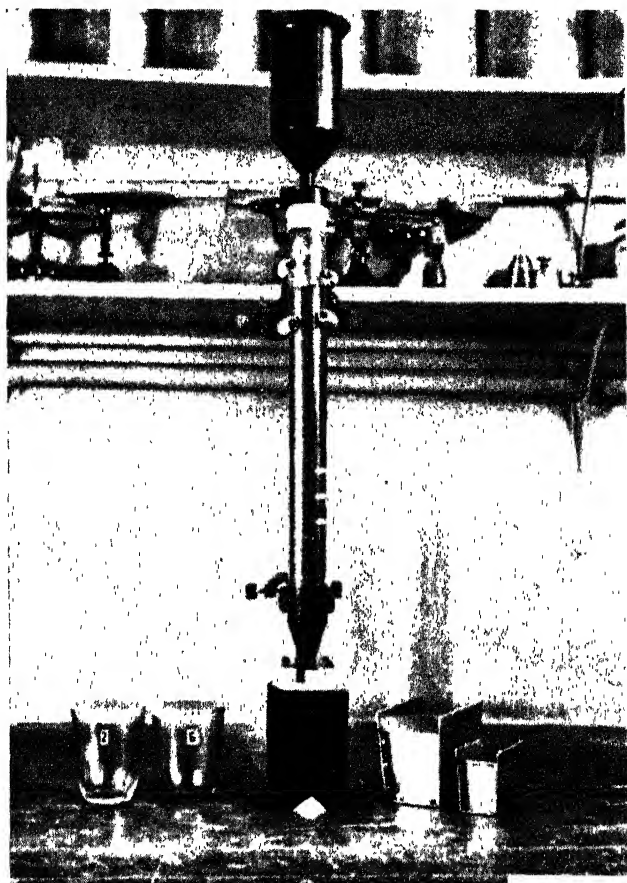


Fig. 4. Miniature loaf volume apparatus.

at 440° F. for 25 minutes. Volumes are measured in a miniature Werner type of apparatus capable of being read to 1 c.c. and shown in Figure 4.

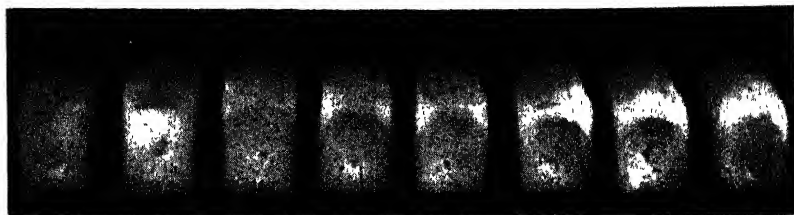


Fig. 5. Fermentation curve using basic 2½% sugar formula.

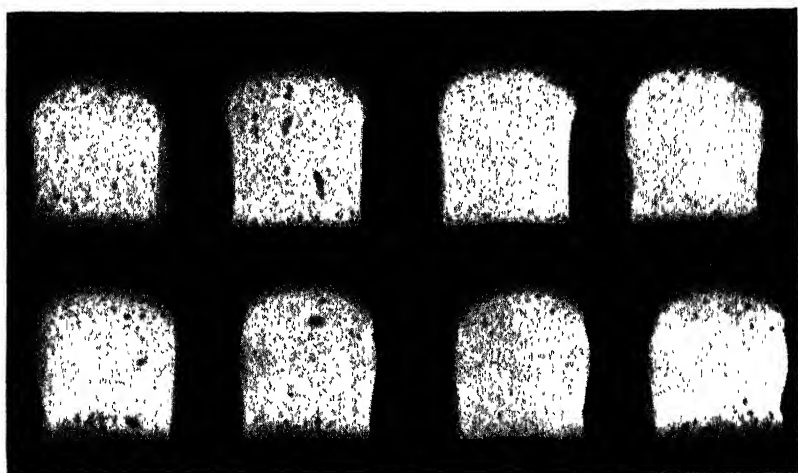


Fig. 6. Cut surfaces of loaves shown in Figure 5.



Fig. 7. Fermentation curve using only flour, water, and yeast.

### Applications and Results

1. Because of the small size of necessary equipment micro baking is a convenient means of making fermentation time-volume curves or other series bakes of that nature. For this purpose the entire

amount of flour is mixed at one time and immediately divided into 40-g. aliquots for fermentation. This procedure eliminates a possible



Fig. 8. Cut surfaces of loaves shown in Figure 7.

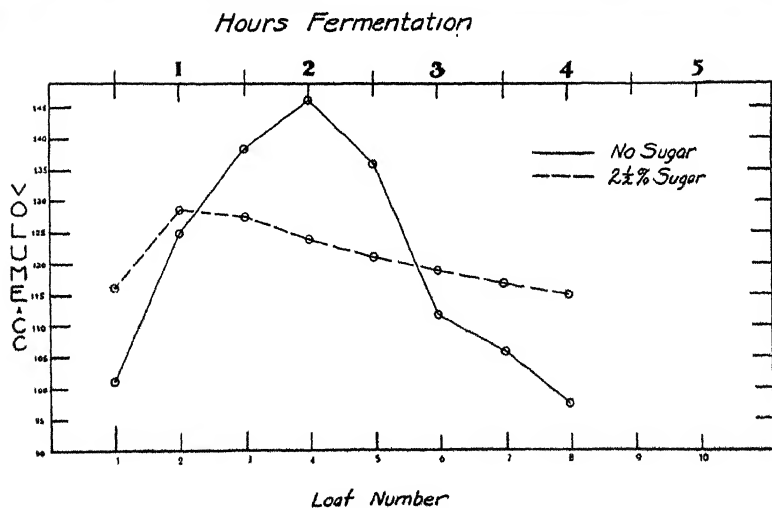


Fig. 9. Graph of loaves shown in Figures 5 and 7.

mixing variable in any one curve. Figure 5 shows a regular basic curve using 2½% sugar. Reading from left to right the times are one-half hour to four hours by half-hour steps. The external forms

of these loaves are as characteristic as loaves of any size would be. Figure 6 shows the internal structure of the same loaves, reading from left to right beginning with the top row. The internal features are

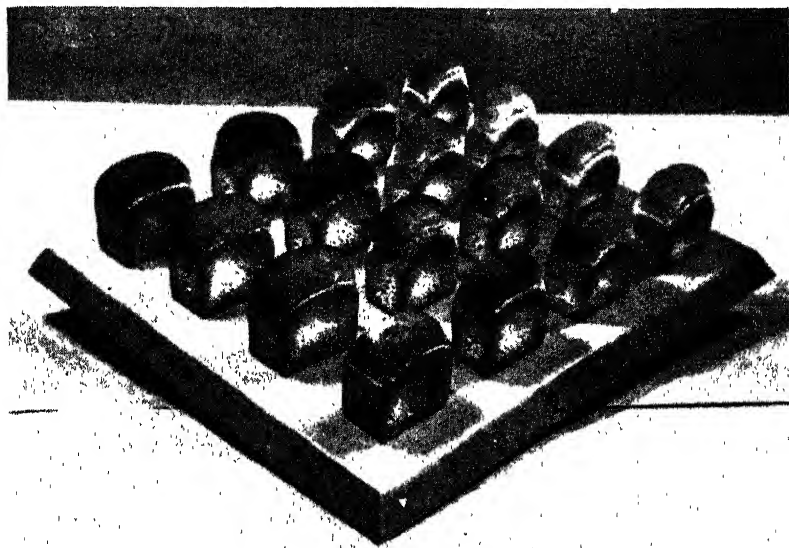


Fig. 10. Checkerboard baking test.

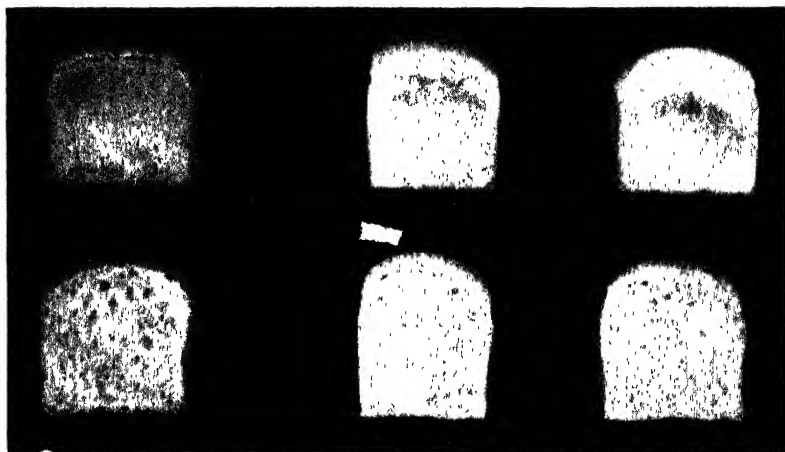


Fig. 11. Representative loaves of checkerboard test (Figure 10).

also as characteristic as they would be in loaves of larger size. Figure 7 shows a sponge development curve made without sugar with fermentation times, left to right, of one-half hour to four hours by half-hour

steps. Figure 8 shows cut surfaces of the same loaves, again reading from left to right beginning with the top row. External and internal features of these small loaves are entirely characteristic. For comparison, Figure 9 shows the two preceding curves in graphical form.

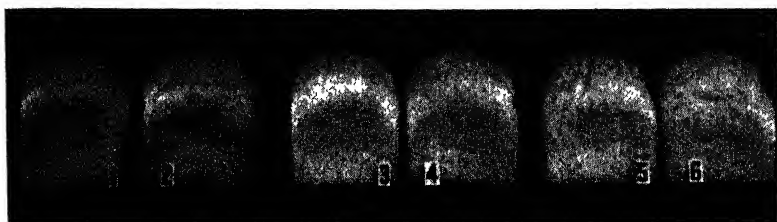


Fig. 12. Routine differential loaves.



Fig. 13. Cut surfaces of loaves shown in Figure 12



Fig. 14. Comparison of 25-g. and 100-g. loaves using two types of flour.

Curve baking gives very informative results and the micro method is particularly adapted to this use.

2. The checkerboard, or Latin square, system of baking proposed by Clark (1937) has attracted attention recently. The micro method



is again very convenient for this type of testing because of the size of equipment and precision of handling. Figure 10 shows a composite picture of the results of such a test, with fermentation times varied

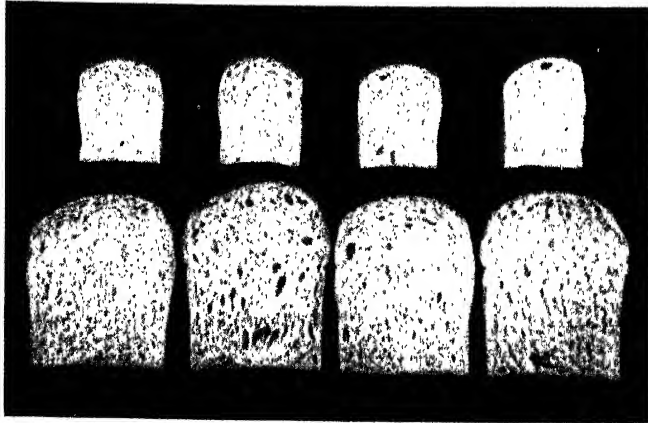


Fig. 15. Cut surfaces of loaves shown in Figure 14.

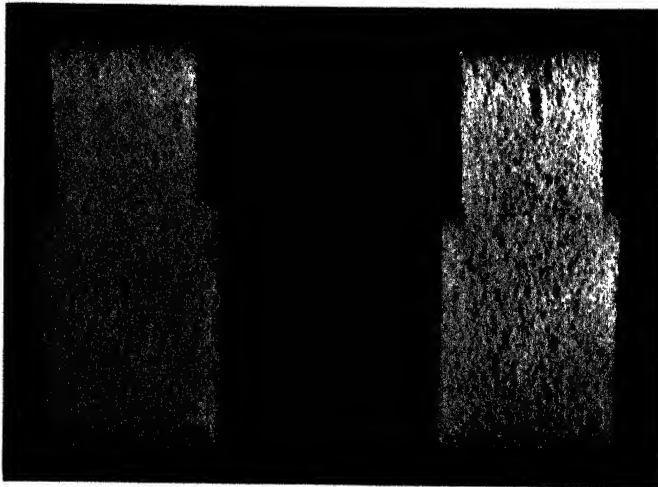


Fig. 16. Enlarged view of sections cut from loaves in Figure 15, eliminating crust.

from one hour to four hours and mixing times from one minute to four minutes. In this case the four loaves representing each mixing time are mixed together and divided into 40-g. aliquots. These 16 loaves are accommodated on a base measuring 15 inches by 17 inches.

Figure 11 shows both external and internal views of the extremes of treatment, together with one of the intermediate loaves. From left to right they are one minute, one hour; 2 minutes, 3 hours; and 4 minutes, 4 hours. The checkerboard system requires a large number

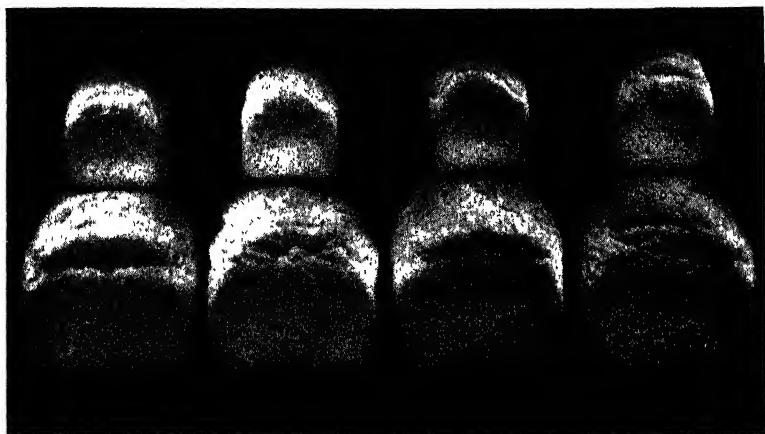


Fig. 17. Comparison of 25-g. and 100-g. loaves in two other types of flours.

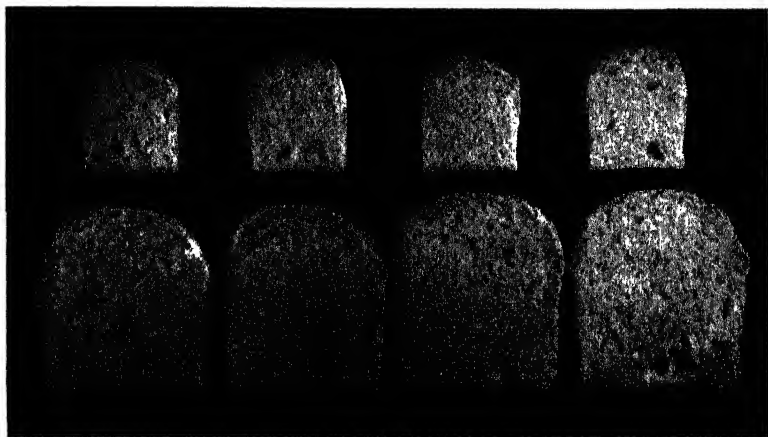


Fig. 18. Internal view of loaves shown in Figure 17.

of loaves and can be nicely handled by this method without excessive-sized fermentation and oven equipment.

3. Micro baking is equally applicable to routine differential baking tests. For this purpose 100 g. is mixed and unused portions discarded. Figure 12 shows external views of three flours of similar type but of decreasing strength from left to right. The even-numbered loaves

are the bromate supplements, all having  $3\frac{1}{2}$  hours of fermentation time. Figure 13 shows internal features of the same loaves. Loaves 1 and 2 have a rather rugged type of grain and 3 and 4 the elongated type, while 5 and 6 have begun to show a little distress, particularly in the plus loaf. Results pictured in both external and internal views can be interpreted as easily as results obtained with larger-sized loaves.



Fig. 19. Further comparison of types in 25-g. and 100-g. formulas.

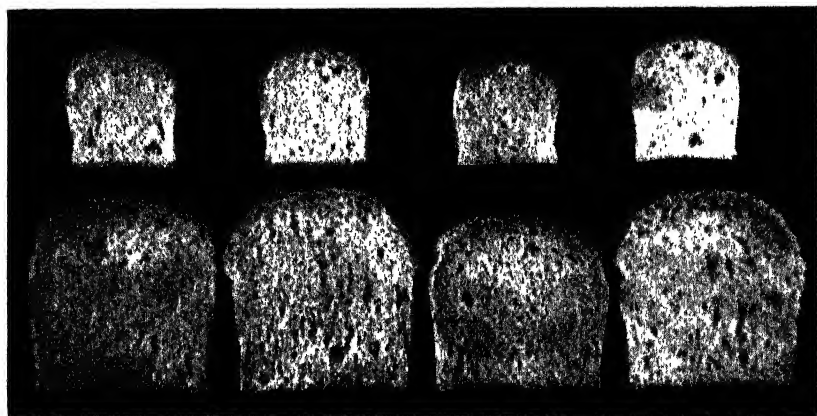


Fig. 20. Cut surfaces of loaves shown in Figure 19.

Figure 14 shows a comparison of two types of flours in 25-g. and 100-g. formulas. The right-hand loaf of each pair is the bromate supplement. The 100-g. loaves were molded by the standard hand procedure; otherwise baking conditions were the same. Figure 15 shows cut surfaces of the same loaves. It is possible that some difficulty encountered in judging small loaves for grain and texture may have been due to the different crust-crumbs ratio. Figure 16 shows

sections cut out to eliminate viewing the crust. The smaller top portion of each pair is from the micro loaf. Variations in grain and texture are within the limits shown by Moen (1935).

Figures 17 and 18 show further external and internal comparisons of different types and Figures 19 and 20 a third comparison. In the comparative pictures all of the flours were of different types or grades. The right-hand set of loaves in Figure 20, for example, are typical of an unbleached flour low in diastase.

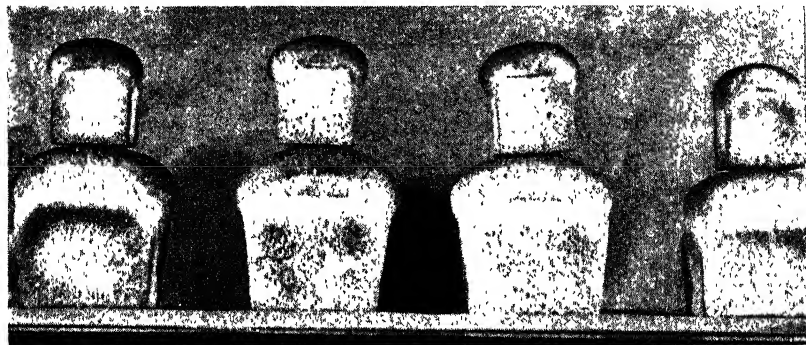


Fig. 21. Comparative results with both sizes of loaves machine molded.

Figure 21 is the only available photograph of a comparison in which both sizes were machine handled. The larger loaves were molded on a commercial molder adapted to 100-g. size. Considerable collaborative work of this kind was done but not recorded photographically and the closing of the collaborating laboratory prevented further work. In this photograph the two sets of loaves show almost identical characteristics.

### Summary

The micro baking procedure is described and the apparatus and typical results and comparisons are shown. The results of the three types of testing described are as informative as results obtained with larger loaves. Quantity baking without excessive-sized equipment, precision, elimination of variables in some cases, and greater ease of replication of results are some of the advantages of the 25-g. procedure. When mechanical equipment is used there is no difficulty in obtaining satisfactory differentiation of both external and internal characteristics.

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## RECORDING-MIXER MEASUREMENTS OF CHANGES IN GLUTEN QUALITY DURING FERMENTATION<sup>1</sup>

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(Read at the Annual Meeting, May 1938)

Recently a paper (Malloch, 1938a) was published describing a recording mixer for small samples. Evidence was presented showing that the breaking time, that is, the time of occurrence of a characteristic break in the downward course of the curve, is probably related to gluten quality. The results of further investigation of this relation are now given.

### Plan of Experiment

Since no satisfactory independent measure of gluten quality of proved validity exists, recourse must be had to its inference from the results of baking tests. However, as these vary considerably with the baking procedure used, it is evident that the relation between loaf volume and the quality of the protein *as it exists in the original flour* must be indirect. The colloidal characteristics of the flour protein will be modified by the addition of other dough ingredients and still further during fermentation. It is useless, therefore, to expect a close relation between the results of baking tests and the results obtained with any kind of mechanical dough-testing device working on flour-and-water doughs, unless (1) the modification of the gluten by the other ingredients and by fermentation is comparable for all flours tested, or (2) the differences in the gluten of the original flours are so great that the variations in modification are masked. The first condition is unlikely to be realized in practice and when the second applies no information on the utility of the machine to distinguish small differences can be obtained. This difficulty can be overcome by making the measurements on fermented doughs at a time corresponding to

<sup>1</sup> Published as Paper No. 147 of the Associate Committee on Grain Research of the National Research Council of Canada and the Dominion Department of Agriculture.

the end of the proofing period, and this was the procedure adopted in this investigation.

Since, with any given oven conditions, the loaf volume will depend on the protein content of the flour, the colloidal properties of the dough at the time of baking, and the gas production, it is possible, by eliminating the effect of variability in protein content and gas production, to assess the colloidal properties by obtaining the loaf volume. In this experiment the effect of differences in the protein content of the flours used was eliminated by studying only the changes in loaf volume and breaking time at varying fermentation times *within* each flour, and the effect of variation in gas production was allowed for by appropriate statistical calculation.

Twenty flours of varying quality milled from United States wheat in a commercial mill were used. Table I gives a description of the

TABLE I  
EXPERIMENTAL MATERIAL

Origin	Class of wheat	Flour protein	Loaf volume
		%	c.c.
Elevator lot	Winter	10.0	462
Elevator lot	Winter	10.7	502
Elevator lot	Winter	11.7	528
Iowa	Winter	11.3	578
Iowa	Winter	11.2	473
South Dakota	Spring	13.7	593
South Dakota	Mixed spring	14.8	582
South Dakota	White	12.4	613
South Dakota	Spring	14.2	554
Kansas	Winter	12.1	608
Kansas	Winter	11.1	543
South Dakota	Spring	14.5	671
Elevator lot	Spring	15.2	684
Minnesota	Spring	12.6	607
North Dakota	Spring	12.8	615
Minnesota	Spring	12.7	671
Minnesota	Spring	13.8	696
North Dakota	Spring	13.0	677
North Dakota	Spring	13.1	625
Elevator lot	Spring	14.0	688

wheats with the protein content of the flour and the loaf volume obtained, using the malt-phosphate-bromate formula and the usual A. A. C. C. procedure and fermentation time.

Each flour was baked by the malt-phosphate-bromate formula at five fermentation times varying from 1 to 5 hours. Recording-mixer tests were made at times corresponding to the end of the proofing periods on successive portions of a dough mixed by the same formula, using the same absorption and fermented under the same conditions. The gas production during the proofing periods was determined by the

Blish method (Blish, Sandstedt, and Astleford, 1932, and Sandstedt and Blish, 1934) by means of an apparatus recently described (Malloch, 1938b).

### Results

The results of the three determinations are summarized in Figure 1, which shows the average values for the 20 flours at each fermentation time. The rough relationship, that loaf volume increases with increasing gas production and with decreasing breaking time, is apparent from this graph.

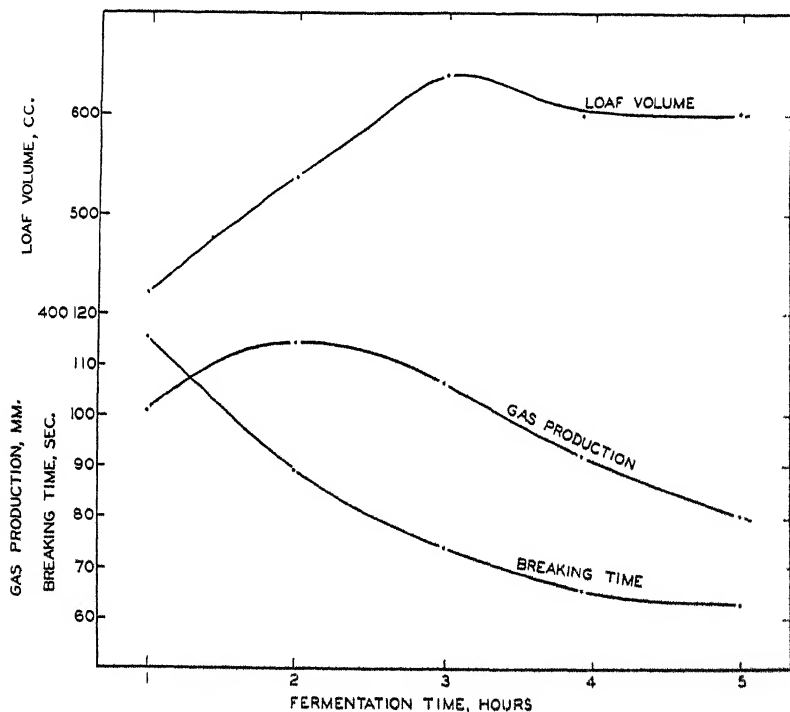


Fig. 1. Average values for 20 flours.

In Figure 2 a series of curves obtained with a single typical flour are shown. These show the characteristic differences between a curve for a flour-and-water dough and one for a complete dough and also the subsequent changes due to fermentation. The corresponding changes in breaking time for the average of the entire series are shown in Figure 3 together with the effect of fermentation on the breaking time of two individual flours. These curves confirm the necessity of comparing the loaf volumes with the breaking times obtained under comparable conditions.

Little information regarding the nature of the relations involved can be obtained by a simple examination of the data and recourse must be had to statistical analysis.

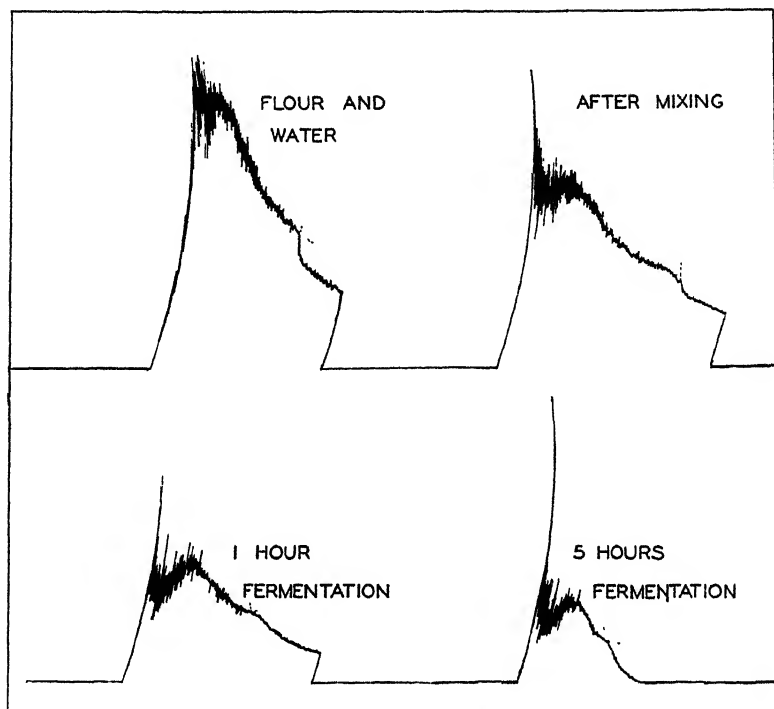


Fig. 2. Curves obtained with a single characteristic flour showing changes due to mixing of usual dough ingredients and fermentation.

### Statistical Analysis

The variance and covariance within flours were computed and used as the basis for the calculation of the partial regression and multiple correlation coefficients relating the changes in gas production for different proofing periods, and breaking time at the end of different proofing periods, with the variation in loaf volume due to difference in fermentation time. The first calculation was made on the assumption that the relations between gas production and loaf volume and between breaking time and loaf volume were both linear. Then a curved relation was assumed for gas production only and a quadratic term inserted in the gas-production portion of the equation. Finally it was assumed that both relations are curved and a quadratic term was



inserted for breaking time also. The results of these calculations are given in Table II.

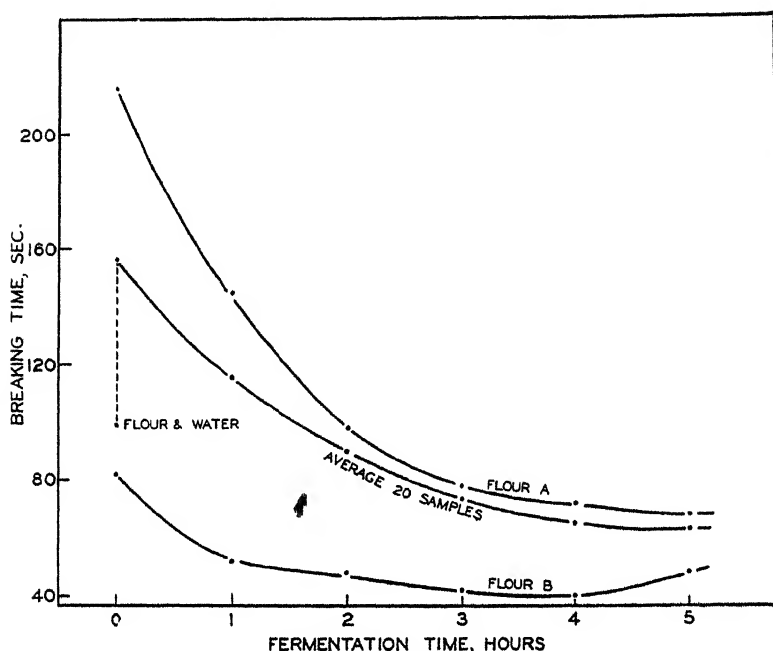


Fig. 3. Average values for the 20 flours and values for two individual flours.

TABLE II

RELATION BETWEEN CHANGES IN GAS PRODUCTION AND BREAKING TIME, AND CHANGES IN LOAF VOLUME WITHIN FLOURS

Partial regression coefficients	Multiple correlation coefficient, $r$
$+ 2.67P - 3.26T$	.84
$+ 33.32P - 0.158P^2 - 3.27T$	.90
$+ 16.04P - 0.060P^2 - 12.50T + 0.036T^2$	.97

P = gas production during proofing period (mm.)

T = breaking time at end of proofing period (sec.)

The proportion of the variance in loaf volume accounted for increased progressively with the insertion of each quadratic term. The significance of these was tested by calculating the residual variance in each case and comparing the variance accounted for by the regression terms with the mean square residual. The F values were 127.1 and 165.7, respectively, while the value of F at the 1% point is 7.0. The curvature of both relations is therefore highly significant.

With the double quadratic equation the residual variation of loaf volume as measured by the standard deviation is only 23.32 c.c. This may be compared with the experimental error of the determination of loaf volume given by the standard error of the mean of replicate determinations (7.82 c.c.). It is possible that better fits could be obtained by the use of higher-order equations, but the residual variance is already so low that the very considerable labor involved in fitting cubic terms does not seem to be justified.

### Discussion

The statistical analysis shows that there is a very close relation between the variation in loaf volume within a single flour at different fermentation times and the changes in gas production and breaking time of similar doughs measured at comparable times. The residual variation in loaf volume unaccounted for by the change in the other variables is only 23 c.c., and even this small error can be partly accounted for by experimental errors in the three determinations involved. Thus it is established that in the series of samples studied the effect of varying fermentation time on loaf volume can be attributed to the changes in gas production during proofing at different periods and to the variation in some factor which is measured by the breaking time determined at the end of the proofing period. The relations involved are both markedly non-linear. As the gas production increases a smaller proportion of the total gas is retained and the curve relating gas production and loaf volume flattens out. Similarly as the breaking time becomes shorter the loaf volume increases and the change is more pronounced with the shorter breaking times.

The samples covered a wide range of flour quality (Table I) and this with the different fermentation times gave a wide variety of baking conditions. The extreme range of loaf volume was from 392 c.c. to 755 c.c. Gas production during the proofing period varied from 71 mm. to 121 mm. and the breaking time at the end of the proofing period from 40 sec. to 210 sec. Since five fermentation times were used with each of the 20 flours, 80 independent estimates of the changes due to fermentation were available as a basis for the conclusions. The very close relation discovered over the entire series shows that each individual flour must have behaved in a similar manner. In view of the wide variety of baking conditions and the similar behavior of all the flours, there is a strong presumption that the relation found in this series will have general application.

The variation in loaf volume within a single flour can only be caused by changes in the gas production and colloidal properties and it has been shown to be a function of changes in gas production and breaking

time. Therefore, the breaking time must be related to the colloidal characteristics of the dough. These colloidal characteristics are determined mainly, if not solely, by the condition of the gluten, generally called "gluten quality." Hence, with respect to the properties affecting loaf volume, the changes in gluten quality during fermentation must be indicated by changes in the breaking time with a high degree of accuracy. It is not suggested that the changes in the properties measured by the breaking time are the only ones taking place during the fermentation of dough. Indeed the character of the recording-mixer curves for various fermentation times is good evidence to the contrary. However, if any of the other properties influence loaf volume, then the changes in them must be closely correlated with the changes in the properties affecting breaking time, which, in actual practice, gives a measure of the combined effect.

Since the changes in gluten quality are measured accurately by changes in the breaking time, the utility of the breaking time of flour-and-water doughs as a measure of gluten quality of the original flour is strongly indicated. However, the ability of the breaking time to distinguish differences between the gluten qualities of different flours must be established before this conclusion in logic can be established in fact. The present series is too short for investigations of this kind, as only 19 effective comparisons between flours are available as contrasted with the 80 on which the present conclusions are based.

The pronounced effect of gas production during the proofing period on loaf volume, which is established in this investigation, gives definite experimental confirmation to the importance which has been generally attached to the gassing power during this phase of baking. It is, of course, particularly important in test baking. In the baking test using the regular A. A. C. C. procedure, which was conducted as part of the experiment, the gas production during the proofing period was 83 mm. for the lowest-gassing flour and 104 mm. for the highest, in spite of the use of high diastatic malt and phosphate in the formula. This difference would be sufficient to account for a difference in loaf volume of approximately 100 c.c. The interpretation of the results of baking tests in terms of the strength of the flour may be seriously in error if differences in gas production of this magnitude are not taken into account. However, in any attempt to equalize the gas production, the continuous modification of gluten quality during fermentation should receive due consideration.

### Summary

The utility of a new recording mixer using small samples for following with a high degree of accuracy the changes in gluten quality during fermentation is demonstrated.

# CEREAL CHEMISTRY

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Proof of the importance of controlling gas production during the proofing period in test baking is given.

### Acknowledgments

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## THERMOSTATIC DOUGH-TEMPERATURE CONTROL FOR HOBART-SWANSON MIXER

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(Read at the Annual Meeting, May 1938)

The factors which influence the mixing of a dough seem to be somewhat complex and of a varied nature. Skovholt and Bailey (1932) have shown that the height of the farinograph curve varied markedly with temperature. Bohn and Bailey (1936) report extensive studies as to the effect of mixing on the physical properties of the dough. Talbott and Weaver (1933) devised an apparatus for the convenient and accurate delivery of solutions used in the experimental baking test. Other cereal chemists have noted the importance of temperature control on dough behavior, and have spent considerable time and study on these factors.

### Procedure

Having access to a thermostat used as a temperature-control unit with the Brabender farinograph, we have limited our investigation to include only the possibility of controlling the temperature of the dough during the mixing stage. The thermostat used was found to be very efficient in water of the desired temperature circulating through a water bath fastened to the mixing bowl of a Hobart-Swanson mixer. Pre-

liminary tests showed that the aluminum mixing bowl is an excellent conductor of heat.

The temperature factors, which may partly or wholly be controlled in the laboratory, vary considerably from day to day, and must be considered in the light of the ultimate results obtained. The temperature of the dough from the mixer is an important factor to consider either in the laboratory or bakery, and is controlled as accurately as the equipment used will permit.

The temperatures considered in this report as affecting the mixing stage were those of the room, flour, water, yeast, salt, sugar, bromate, and any other ingredient added in either a dry or a liquid state. Solutions of the ingredients are made to conform to those specified in the A. A. C. C. experimental baking test, which greatly facilitate temperature readings.

It was deemed advisable to take the temperature of the solutions and ingredients just prior to mixing, and to repeat this procedure at

TABLE I  
TEMPERATURE INCREASE (F) OF SOLUTIONS IN MIXING BOWL

Temperature of room, water, and solutions used in A. A. C. C. exp. baking test <sup>1</sup>	Thermostat temp.	Temperature of solutions in mixer at time intervals (minutes)			
		1½	3	4½	6
<i>Av. ° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>	<i>° F.</i>
77.2	84.2	82.0	82.5	82.5	83.0
77.2	86.0	83.5	83.5	84.0	84.5
77.9	87.8	86.0	86.5	86.5	86.5
79.0	89.6	87.0	88.0	88.0	88.0
79.0	91.4	88.0	88.0	88.5	89.0
79.0	93.2	89.0	90.0	90.0	90.5

<sup>1</sup> Water based on 65% absorption.

TABLE II  
DOUGH TEMPERATURES UNDER THERMOSTAT CONTROL  
IN A HOBART-SWANSON MIXER

Flour No. <sup>1</sup>	Water	Mixing time of dough	Temperature of room, water, and solutions added in A. A. C. C. baking test	Thermostat temp.	Dough temp. from mixer
	%	<i>Min.</i>	<i>Av. ° F.</i>	<i>° F.</i>	<i>° F.</i>
1	60	1½	79.0	86.0	85.0-85.0
2	64	2	75.0	89.6	85.0-85.0
3	65	2	79.0	87.8	86.0-87.0
4	66	2½	78.0	87.8	86.0-86.0
5	65	2½	78.0	87.8	85.0-85.0
6	65	2½	77.0	87.8	85.0-85.0
7	65	2½	79.0	87.8	86.0-86.0
8	68	3	78.0	87.8	87.0-86.0

<sup>1</sup> No. 1—weak family flour. Nos. 2, 3, 4, 5—hard winter wheat flours. Nos. 6, 7—spring wheat flours. No. 8—spring clear flour.

definite time intervals. Any appreciable change in the temperature of the solutions added would of course necessitate a thermostat correction, all other factors being constant. In order to obtain if possible a more

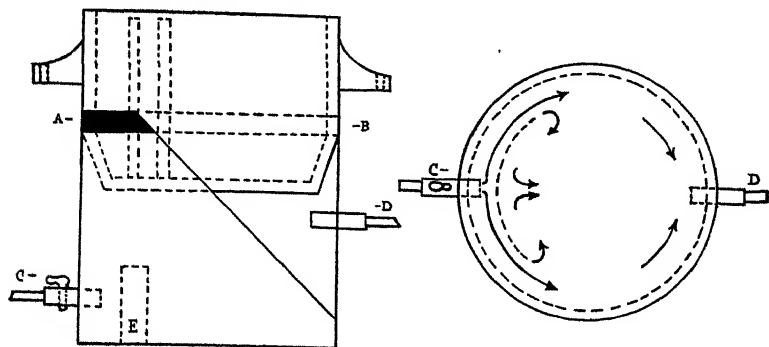


Fig. 1. Diagram showing water bath fastened to bowl of Hobart-Swanson mixer. *A*, steel band supporting bath to mixing bowl. *B*, rubber facing to prevent leakage. *C*, inlet. *D*, outlet. *E*, curved fin.



Fig. 2. View showing Hobart-Swanson mixer and thermostatic temperature control.

comprehensive picture as to the degree in which the thermostat indicates temperature changes of the solutions in the mixer, a series of tests were made at intervals of one and one-half minutes (Table I). The designated interval of time approximated somewhat closely the laboratory mixing schedule.



The flours which are reported in Table II were chosen at random from data accumulated over a considerable period of time, and under temperature variations experienced in the laboratory on that particular day. Both hard-winter and spring wheat flours are listed, using a variable mixing time and absorption (500 units) approximating that obtained on the Brabender farinograph.

### Discussion

From the data shown and accumulated over several months of continuous operation, it would seem that thermostat control has some value in mixing. The results do not seem to indicate that any appreciable heat is generated in mixing, which is probably due to the even temperature of the surfaces with which the dough comes in contact. There seems to be a certain loss of heat due to radiation in the mixer and from the tubing leading to the thermostat, which accounts for the lower temperature reading of the solutions in the mixer.

The order in which the solutions are added to the mixing bowl seems to influence the temperature of the resultant dough. The distilled water, being the chief ingredient in terms of volume and likewise with few exceptions the coldest, was placed in the mixer first. This was followed by adding the salt and sugar, bromate, and yeast solutions. A thermostat temperature range of 30° to 33° C. has been found applicable to existing temperature variations from day to day. During the extremely warm weather of the summer months, the thermostat has been operated successfully to control the dough temperature. The relatively short time that it takes to increase or decrease the temperature of the thermostat makes it a very useful piece of equipment in the laboratory.

### Summary

A thermostat control may be used effectively in controlling the dough temperature in the mixer. The accuracy of the control will permit results varying not more than  $\pm 1^\circ$  F. from the dough temperature desired.

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## REPORT OF THE 1937-38 COMMITTEE ON STANDARDIZATION OF LABORATORY BAKING <sup>1</sup>

C. F. DAVIS, *Chairman*

Western Star Mill Co., Salina, Kansas

(Read at the Annual Meeting, May 1938)

During the past year the committee has endeavored to carry on work along lines recommended by the 1936-37 committee. It appears that some definite progress has been made but the work of the Committee on Standardization of Laboratory Baking is not to be measured in terms of a year's work.

Development work on all test baking equipment is in progress. The development of sheeting and molding equipment has been encouraged to the extent that two papers are offered for this program relating to the possible value of such equipment. Manufacturers have been encouraged to the extent that we have exhibited at this convention some sheeting and molding machines which are available at moderate cost and which are definite improvements over hand operations. In order to cooperate with manufacturers and facilitate immediate further development of molding equipment this committee has unanimously agreed to establish a 10 cm. bottom pan length with the idea of offering next year for the approval of the association an arbitrary pan built around this dimension and suitable as an optimum pan size for the loaf volume range of from 550 to 750 c.c.

Two contributions on the subject of sponge dough laboratory baking tests are offered on this program and further contributions are in the process of development.

This committee would recommend that further work be done as recommended by the 1936-37 committee.

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<sup>1</sup> General report.

# HAND PUNCHING AND HAND MOLDING VS. MACHINE PUNCHING AND MACHINE MOLDING <sup>1</sup>

W. L. HEALD

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(Read at the Annual Meeting, May 1938)

For the past few years the Baking Committee has recognized the problem of standardized equipment for the successful operation of the baking test if it is to continue as the official baking test of the American Association of Cereal Chemists.

## Previous Investigations

G. Moen (1929) published results of the Baking Committee's work for that year in which he found between two operators a difference of 5.4% in loaf volume, while the grain and texture also were manifestly different. In the interest of securing more uniformity through mechanization, the Thompson Machine Company developed a one-man molder, type "I." Fifield and Weaver (1930), of the U. S. Department of Agriculture, secured the use of one of these machines and reported that whereas Moen had experienced a 5.4% difference in loaf volume with a molding machine and different operators, a variation of 1.9% was experienced in their work. Thus it would seem that a step had been made in the right direction.

Merritt and Blish (1931) carried out additional work with a Thompson molder. Their conclusions were that while the machine gave greater uniformity than hand molding, they still encountered some variations which they were unable to overcome in any adjustment made on the machine. The same year Geddes and Goulden reported an extensive study of the variability as influenced by mechanical molding involving more than 4,000 loaves in which the personal factors of punching and molding were studied. Result of this work was as follows: "Hand molding does not appear to be a factor of major importance in causing variability between replicate bakes. Since punching and molding personality both contribute to the variability between bakers, the introduction of mechanical molding machines may be expected to reduce but not eliminate the large difference in mean loaf volume which different operators working in the same or different laboratories secure in replicate bakings of the same flour."

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<sup>1</sup> Sub-committee report, 1937-38 Committee on Standardization of Laboratory Baking.

Merritt, Blish, and Sandstedt (1932), on further work of the A. A. C. C. fellowship with machine molding, found that the personal factor in dough punching was perhaps the cause of the greatest variation. In view of this fact, a pair of sheeting rolls were devised called the "S" rolls. These rolls operated by hand and, as reported in the work, tended to reduce variation. Geddes and Sibbit (1933) also reported that the use of the "S" rolls very definitely tends to reduce variability between operators.

Freilich (1933) also reported that sheeter molding has a tendency to produce a smaller variation than hand molding and that the sheeter molder gave no better concordance among the mean volumes obtained with different flours than hand molding. A further report of Freilich (1935) with a motor-driven sheeter was as follows: "The sheeter is decidedly to be preferred over hand molding if one wishes to obtain better results when comparing different samples on a given day in his own laboratory. Molding by hand is to be preferred over the sheeter when better agreement between different laboratories is desired."

### Discussion of Sheeter and Molder

The writer had constructed a pair of "S" rolls in 1934 as outlined in *Cereal Chemistry*, 9: 195, except that they were motor driven. Some experimentation as to the speed of the rolls was carried out and a speed of 150 r.p.m. was found to be satisfactory.

Having definitely established a procedure, we have used the method for the past four years in all our punching and sheeting prior to hand molding. VanScoyk (1937) described a molder devised to mold micro loaves very satisfactorily. The writer, believing that a molder designed to handle the A. A. C. C. baking procedure would be very desirable, decided to construct one for his own use. Since we had the sheeting rolls in operation, it was decided to build the apparatus as outlined by VanScoyk, that is, the molder itself separate from the sheeter. It is not a question of speed in the laboratory molding operation, but one of precision, and it was thought that perhaps some of the variation might be eliminated by curling or rolling the doughs by hand between the operations with the sheeter and the molder.

Since we had no data as to the diameter or the width of the drum which would give satisfactory results we had to carry out a series of bakes using different widths of drums and compression plates. At first we decided on a drum 16 inches in diameter and  $3\frac{1}{2}$  inches wide. With an adjustable compression plate, we tried a great many settings but were never able to get a satisfactorily molded loaf that fit the A. A. C. C. tall-form pan. We did, however, get a loaf molded satisfactorily for the low-form pan.

Since the sides of the compression plate were adjustable we decided to try a drum 16 inches in diameter and 5 inches wide on the face. This, of course, necessitated making a new compression plate. This drum and plate we have found to give excellent results with the following compression plate settings.

The entrance end is set  $1\frac{1}{2}$  inches from the drum and at the discharge end 1 inch from the drum. Slots are cut in the side plates so that adjustments can be easily made. The speed of the drum is 30 r.p.m. Both faces of the drum and the compression plate are covered with cotton belting.

### Operation of Molder

The procedure in connection with the molder is as follows: A. A. C. C. formula and fermentation time. At punch time the dough is removed from the fermentation bowls as carefully as possible. The wet edges are folded together, and the ball of dough is elongated slightly and placed through the sheeter rolls, lightly rolled up and again placed in the fermentation bowl.

The second punch is the same as the first. At pan time the dough is again removed and passed through the sheeting rolls. This time the dough is laid on a board covered with cotton belting and again rolled the same as in the punching procedure. The lightly rolled dough is placed in the molder with the loose end or flap as far away from the revolving drum as possible so that the seal will not have a pocket of occluded air and show large holes in the baked loaf. When the molded dough comes from the molder it is slightly longer than the pan but the dough draws up slightly during the placing in the pan, which we find just fills the pan nicely. If it should have a tendency to hang on the ends, a flat spatula places it in position very easily. In fact we have found it a good practice to use a spatula on all the loaves. Since doing this we have practically no loaves with low or bulging ends.

### Discussion and Summary of Results

To see if a more consistent baking procedure could not be established by use of the sheeting rolls and the molder, a series of daily bakes were carried out in duplicate extending over a period of a month. Each day a series of eight loaves were baked as follows and illustrated in Table I:

- A—machine punched and machine molded
- B—machine punched and hand molded
- C—hand punched and machine molded
- D—hand punched and hand molded

TABLE I  
AVERAGE LOAF VOLUMES IN CUBIC CENTIMETERS OF DUPLICATE BAKES

No.	Machine punched, machine molded A	Machine punched, hand molded B	Hand punched, machine molded C	Hand punched, hand molded D
1	551	512	515	478
2	527	521	515	490
3	539	506	521	509
4	536	512	533	524
5	521	509	512	492
6	527	527	536	512
7	542	521	498	490
8	527	506	507	521
9	518	493	504	488
10	515	495	495	500
11	527	521	506	521
12	539	506	515	496
13	525	500	509	495
14	527	490	536	506
15	542	542	551	545
16	533	509	553	524
17	512	485	521	488
18	521	515	504	485
19	527	515	521	518
20	542	524	506	512
21	530	509	515	506
22	548	509	539	506
23	527	509	509	485
24	521	506	495	492
25	551	530	527	524
Mean volume value	531.0	510.84	517.88	504.36
S. E. of duplicate determination	9.85	9.28	11.88	11.60
Standard deviation	10.89	13.00	16.11	16.54
Range	39	57	58	67

We find from Table I that the mean volumes of the machine-punched and machine-molded loaves were consistently larger, and also that the standard deviation as well as the range is smallest for the machine-punched and machine-molded loaves.

While loaf volume as reported in cubic centimeters was of most importance in this series of tests, it was also noted that the more uniform grain and texture were to be found in the machine-punched and machine-molded loaves. In other words, the machine-punched and machine-molded loaves had fewer holes and practically no large holes in any of the bakes made, while the hand-punched and the hand-molded loaves tended to have slightly less uniform grain and texture.

We find also that the standard error between duplicates is slightly lower in the machine-punched and hand-molded loaves, the "B" series. The reason for this is not particularly evident. The range of loaf volume between duplicates is less in the case of the machine-

punched and machine-molded loaves and greatest in the hand-punched and hand-molded loaves. It is interesting to note that the hand-punched doughs, namely "C" and "D," are considerably higher in standard deviation than "A" or "B." This tends to substantiate data already reported to the effect that as a personal factor the punching is more of a deciding factor than the molding.

It was impossible for us to carry out a series with an inexperienced operator, but it is believed, as has been reported by other workers, that the machine-punched and machine-molded loaves would, with unskilled operators, materially reduce the variation to be expected.

Results as here illustrated are not as much in agreement as we had hoped for. We feel, however, that machine punching and machine molding are to be desired over hand punching and hand molding.

### Statistical Discussion

Using four handling methods, loaves were baked in duplicate on each of 25 successive days, the same previously aged flour being used throughout. An analysis of variance was run on the data, and the interaction thus obtained was found to be significant as compared with the duplicate error, and consequently was used as a basis of comparison for the variances attributable to days and handling methods. In addition, the standard errors for duplicate determinations for each handling method were calculated. These were 9.85 c.c., 9.28 c.c., 11.88 c.c., and 11.60 c.c., for methods A, B, C, and D respectively (Table I).

The day-to-day fluctuations were reflected in the standard deviations of the means of duplicates for each of the four handling methods. The differences between days have already been demonstrated to be significant, but it was also desired to know whether or not the variabilities within each method were also significantly different. This was done. The variability in the A method was significantly less than in either C or D, although not less than in B. This would further indicate the greater effect of the punching as a personal factor than the molding.

Considering all the statistical results it would seem that method A is the most desirable. It gives the largest loaf volume, has a small duplicate error, and may be more closely replicated in different bakes as shown by the lower daily variability.

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## COOPERATIVE TEST OF A PUNCHING AND MOULDING MACHINE<sup>1</sup>

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(Read at the Annual Meeting, May 1938)

In 1935 a paper (Malloch and Hopkins) was published describing briefly a machine for punching and moulding 100-g. doughs and giving the results of preliminary investigation of its utility. Since that time a new model of the machine has been built with refinements in the constructional details. This model was tested cooperatively in four Canadian laboratories.

### Description of Experiments

*Experiment 1.* Duplicate samples of fifteen commercially-milled flours, varying in protein content from 10.4% to 15.1% were baked on eight days in each of four laboratories. The order of baking was randomized for each day. One of each pair of doughs was punched and moulded by hand and the other by machine.

*Experiment 2.* Thirty loaves of a single flour were baked on each of four days in each laboratory and alternate doughs were punched and moulded by machine and by hand.

Every effort was made to have uniform conditions in the cooperating laboratories. The same Hobart-Swanson mixer was used throughout, with a mixing time of  $1\frac{1}{2}$  minutes. The moisture contents of the flours and the correct absorptions were determined by one of the laboratories. The flour was shipped in moisture-proof containers and

<sup>1</sup> Published as Paper No. 140 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.  
Sub-committee report, 1937-38 Committee on Standardization of Laboratory Baking.



each laboratory used the specified absorptions. The malt-phosphate-bromate formula was used with the A.A.C.C. baking procedure and all laboratories used low-sided pans. Three extra loaves were included at the beginning and end of each day's baking and discarded. All the laboratories did at least two days of trial baking using the machine before the experiments were started.

### Results and Discussion

The results of an analysis of variance of the loaf volumes obtained in Experiment 1, expressed as standard deviations, are shown in Table I and the average loaf volumes for each laboratory are given in Table II. Corresponding results from Experiment 2 are given in Tables III and IV.

TABLE I  
VARIABILITY OF LOAF VOLUME, EXPERIMENT 1

Lab.	Standard deviation, <i>c.c.</i>			
	Between days (7 D.F.)		Error (98 D.F.)	
	Hand	Machine	Hand	Machine
A	5.4	8.0	16.3	13.8
B	10.6	6.0	28.2	24.4
C	13.6	9.5	23.0	22.8
D	8.5	19.3	43.0	45.2

TABLE II  
MEAN LOAF VOLUME, EXPERIMENT 1

Lab.	Hand	Machine
	<i>c.c.</i>	<i>c.c.</i>
A	794	713
B	789	751
C	747	703
D	712	649

In Experiment 1 laboratories A and B showed significantly lower error by the machine. The differences in the other laboratories were not significant. The differences between days show no advantage for either method of manipulation. The spread between the average volumes is not reduced by use of the machine.

In Experiment 2 all the laboratories gave lower standard deviations "within days" by machine but the differences are only significant for laboratories A and B. The small number of degrees of freedom for "between days" (three) makes it impracticable to compare the standard deviations statistically but three out of the four laboratories

TABLE III  
VARIABILITY OF LOAF VOLUME, EXPERIMENT 2

Lab.	Standard deviation, c.c.			
	Between days (3 D.F.)		Within days (56 D.F.)	
	Hand	Machine	Hand	Machine
A	6.4	3.1	17.8	10.1
B	10.0	14.8	25.0	20.4
C	18.9	6.2	26.0	22.4
D	17.2	16.1	51.7	45.2

TABLE IV  
MEAN LOAF VOLUME, EXPERIMENT 2

Lab.	Hand	Machine
	<i>c.c.</i>	<i>c.c.</i>
A	929	791
B	936	857
C	796	757
D	761	620

gave lower results for the machine. Again, the use of the machine did not reduce the spread between laboratories.

The results show that on the whole there was slightly lower variability when the doughs were manipulated by machine, but the advantage is of no practical importance and certainly does not warrant the general adoption of the machine. This study confirms the conclusion reached in an earlier study of the Thomson moulder (Geddes, Larmour, and Malloch, 1936), that "differences in the manual manipulation of doughs during moulding are relatively unimportant in relation to the total effect of other factors causing variation between replicates."

It is interesting to note, however, that the machine showed to the best advantage in laboratory A, where the variability by both methods was strikingly lower than in the other laboratories. From this it might reasonably be concluded that when the major causes of variation have been removed, mechanical punching and moulding can be usefully employed in test baking. In laboratories where the average loaf volume is low (under 700 c.c.) it might be useful even now.

### Acknowledgments

I am greatly indebted to Dr. W. F. Geddes, Dominion Grain Research Laboratory, Winnipeg; Dr. R. K. Larmour, University of Saskatchewan, Saskatoon; Dr. A. G. McCalla, University of Alberta,

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## A CRITICAL STUDY OF A "PUP" SPONGE BAKING METHOD<sup>1</sup>

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(Read at the Annual Meeting, May 1938)

One of the criticisms often expressed against the American Association of Cereal Chemists' recommended baking procedure is that it is a straight dough method. It is frequently argued that since more of the baked goods sold on the market result from the sponge-dough process than from the straight dough process, it would be advantageous, or possibly essential, to arrange the Association baking test on a sponge-dough procedure. This contention is certainly worthy of every consideration; consequently, the following investigation was undertaken in an effort to formulate a satisfactory "pup" sponge baking method and to evaluate the results obtained.

### Method

The following formula and method were adopted.

<i>Sponge</i>		<i>Dough</i>	
Flour	70.0%	Flour	30.0%
Yeast	1.1%	Milk powder	2.8%
Shortening	1.5%	Salt	2.0%
Malt powder, 20° L.	0.6%	Sugar	2.0%
Arkady	0.3%	Water—sufficient to yield proper dough consistency.	
Water—sufficient to yield proper sponge consistency.			
Sponge time 4 hours.			

Total flour weight was 200 g. on a 15% moisture basis. Both the sponge and dough were mixed two minutes in the Swanson mixer. The dough was fermented 20 minutes, divided into two equal portions, rounded up, allowed to rest 15 minutes, and panned. The proofing time was 55 minutes.

The sponge-dough formula adopted was purposely arranged to include the ingredients used by the average baker. This procedure is

<sup>1</sup> Sub-committee report, 1937-38 Committee on Standardization of Laboratory Baking.

in contrast to the A. A. C. C. baking method, the basic procedure of which utilizes a lean formula with a minimum of ingredients.

Choosing the proper sponge time is always a controversial matter; therefore, the four-hour period selected for this work was adopted, primarily because it came within the range of sponge times generally recommended, and in addition had the advantage of allowing a series of bakes to be completed within the period of a normal working day.

When undertaking to utilize the sponge-dough method to evaluate a series of flours which vary in character over the complete range encountered in the bake shop, it obviously is not a fundamentally sound procedure to attempt the use of the same flour for both sponge and dough purposes. The only justification for such a procedure must be based on the idea that the final baking results possibly would be indicative of the flour's inherent ability to produce bread under the conditions imposed. Embracing this concept invalidates the basic cause for seeking a "pup" sponge-baking method, because the A. A. C. C. procedure, admittedly, even by its most severe critics, does definitely differentiate between flours. Therefore, if a more essential question than this is not sought no useful purpose is served by deviating from the present official straight dough method. In other words the concept is that there already existed a tried and tested baking procedure; consequently, if there is any justification for a supplementary "pup" sponge method of flour testing, then the procedure should parallel closely that encountered in the baking industry. Most assuredly it would not be a sound practice to test all flours by utilizing them for both the sponge and dough. Consequently, to avoid this situation a standard topping flour of known suitability for this particular purpose was used throughout the investigation. Conversely it would be possible by this baking procedure to test the relative suitability of a flour for doughing purposes by using a standard flour for the sponge.

### **"Pup" Sponge-Baking Results**

Figure 1 indicates the general characteristics of "pup" sponge bread baked by the method previously outlined. Bread made from three very different flour types is represented in the picture. From left to right the flour types represented are: a southwestern baker's standard patent, a spring wheat short patent, and a very strong spring wheat straight grade flour. It is evident from Figure 1 that bread of satisfactory internal and external appearance is produced.

### **Comparison of Sponge and Straight Dough Bread**

In order to compare the results of testing flour by both the sponge-dough and straight dough methods, 24 samples of flour of various types

were collected from commercial bake shops. These flours were baked simultaneously by both methods and therefore the physical conditions as well as the ingredients used were identical. The volume of the

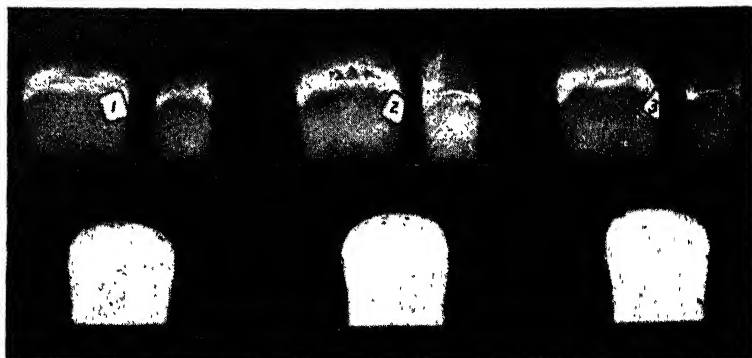


Fig. 1. Examples of "pup" sponge loaves baked from (1) southwestern baker's standard patent, (2) spring short patent, and (3) strong spring straight grade.

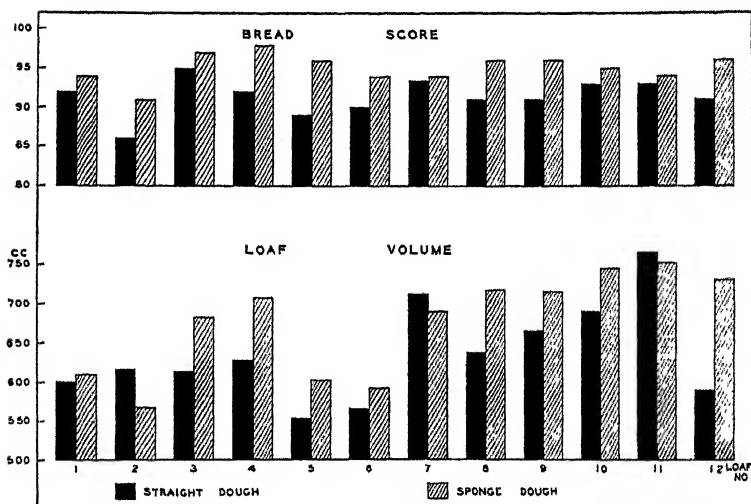


Fig. 2. Typical examples of the relationship between the bread scores and the loaf volumes of bread baked by a straight dough as compared with a sponge-dough method.

baked loaves and the bread score were used as criteria of the differences between the two methods of making bread. The loaf volumes were obtained as soon as the loaves were cool, and the bread was scored the following day. Numerical values were assigned to such internal and external characteristics as volume, symmetry, bloom, break, color, grain, and texture.

A comparison of the two baking methods, by utilizing the 24 flours previously mentioned, revealed a low but significant ( $r = 0.53$ ) correlation between the loaf volumes and a very significant relationship between bread scores. The correlation coefficient in the latter case was  $r = 0.81$ . These relationships are shown graphically in Figure 2.

### Discussion of Results

Loaf volume is only one of the several criteria indicating flour quality; therefore a significant relationship between the loaf volumes of bread baked from the same flour, by both the sponge and straight dough methods, is of limited importance. However, the high correlation between the bread scores when bread is made by both methods is vitally important. Obviously this indicates that both methods are applicable for the evaluation of flour quality. The question then becomes one of determining which one of the two methods, as thus conducted, yields the most valuable results.

The sponge-dough method, in the authors' opinion, is definitely a less sensitive procedure than the straight dough method. Many of the evident differences between loaves which are extremely helpful in evaluating flour baked by the straight dough process are so modified by the sponge-dough method that the tendency is toward a uniform pattern. Thus, from a testing point of view, the sponge dough method is less useful than the straight dough process, and accordingly does not warrant consideration as a substitute for the present "official" baking test.

### Summary and Conclusions

A "pup" sponge baking procedure has been outlined.

Baking comparisons between "pup" sponge and "pup" straight dough methods reveal that there is a significant correlation between the loaf volumes and bread scores obtained.

The sponge-dough method, under the conditions adopted in this investigation, was a less sensitive test of flour quality than the straight dough method.

## THE QUESTION OF SUGAR LEVELS IN LABORATORY BAKING<sup>1</sup>

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(Read at the Annual Meeting, May 1938)

Admittedly an essential feature of any *standard* experimental baking test is the establishment of rational conditions whereby all flours may be tested on an equal basis. Flours vary widely in their gas-producing potentialities, but since the measurement of this factor—which is easily controlled in industrial practice—is not the primary objective in test baking, it should be removed from the list of variables, as has been suggested by numerous workers. As recently pointed out in a detailed discussion by Landis and Frey (1936), the strict elimination of gas production as a variable factor demands a special sugar-level adjustment for each individual flour, if other factors are to be kept constant.

That a properly conducted gassing-power test, involving actual yeast fermentation, provides a far more reliable basis for the measurement of sugar requirements than does the autolytic maltose test is quite generally recognized by cereal technologists. However, recent studies by Blish and Sandstedt (1937) and by Sandstedt and Blish (1938) indicate that the control of sugar levels in doughs does not necessarily insure a corresponding control of uniformity in *rate* of gas production. These studies show that flours contain, in varying degrees, an unidentified biocatalytic "activator" (Factor M), which stimulates and influences rate of maltose fermentation by baker's yeast. This variation in quantity of Factor M seemingly accounts for the observed fact that flours having the same *maltose-producing* potentialities sometimes show significant variations in gas-production rate, and these variations frequently register conspicuously during the dough proofing period.

Since suitable means for controlling the quantity and activity of Factor M are lacking, it follows that rate of gas production is difficult, if not impossible, to control with any high degree of precision. This situation has led Sandstedt and Blish (1938) to the belief that for practical purposes the factor of variable activator content can best be handled

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by insuring an adequate sugar *supply* and then by proofing doughs to constant *height* rather than to constant *time*.

Under certain practical conditions of routine laboratory baking it is inconvenient and time-consuming to undertake to adjust all flours to precisely the sat. gas-production level by means of careful and accurate gassing-power tests. Indeed there are doubtless many purposes and occasions for which such precise control of conditions is quite unnecessary. The first consideration, of course, is to insure against any serious deficiency in gas production by the use of sufficient sugar in the formula. This brings up a question as to what constitutes a serious sugar deficiency, and what quantity of sugar may be regarded as excessive. Within what range can the quantity of sugar be varied without seriously complicating either baking behavior or interpretation of results? Is this range substantially the same for all flours, or does it vary significantly with different flours? In an attempt to secure practical information relative to these and associated matters some experiments were undertaken in which several commercial flours of various type and origin were experimentally baked under conditions involving different sugar levels.

### Experimental

The main objective in the experiments here reported was to test the idea that, when there is no pronounced *deficiency* of sugar supply in the dough, it may be possible to vary the sugar level over a considerable range without seriously affecting loaf characteristics and interpretation of results, provided doughs are proofed to constant *height* instead of to a standard time. The experiments involved six commercial baker's flours whose origins and properties, respectively, are shown in Table I.

TABLE I  
SOURCES AND PROPERTIES OF FLOURS

Flour origin	Protein	Maltose value	Gassing power <sup>1</sup>
	%	mg.	mm. of mercury
1 Pacific Coast	11.2	195	388
2 Montana	14.2	250	412
3 Kansas	12.9	335	416
4 Minnesota	12.9	287	413
5 Kansas	12.5	354	445
6 Canada	13.2	378	545

<sup>1</sup> Total pressure at 4 hours.

Each of the flours shown in Table I was baked, following the A.A.C.C. method, with four different amounts of added sucrose, namely, 2.5 g., 3.5 g., 4.5 g., and 5.5 g., respectively, and all doughs



were proofed to the standard time of 55 minutes. The entire program was then repeated, but all doughs were proofed to standard height instead of standard time. On the basis of preliminary tests, 9.5 cm. was selected as a suitable height. Loaf volumes for the two series of tests are shown in Tables II and III, respectively. The detailed presentation of additional baking data is omitted because such additional data would contribute nothing of importance to a rational interpretation of results.

TABLE II

LOAF VOLUMES FOR VARIOUS SUGAR LEVELS WHEN PROOFING TO STANDARD TIME (55 MINUTES)

Flour	2.5 g. sucrose		3.5 g. sucrose		4.5 g. sucrose		5.5 g. sucrose	
	Proof ht.	Vol.	Proof ht.	Vol.	Proof ht.	Vol.	Proof ht.	Vol.
	cm.	c.c.	cm.	c.c.	cm.	c.c.	cm.	c.c.
1	8.8	535	9.0	545	9.3	595	9.5	545
2	9.8	630	10.1	625	10.0	625	10.1	670
3	8.8	525	9.0	535	9.2	345	9.3	545
4	8.9	570	8.9	560	9.2	570	9.3	615
5	9.0	560	9.0	565	9.1	570	9.1	580
6	10.5	605	10.2	570	10.0	570	10.0	575

TABLE III

LOAF VOLUMES FOR VARIOUS SUGAR LEVELS WHEN PROOFING TO STANDARD HEIGHT (9.5 CM.)

Flour	2.5 g. sucrose		3.5 g. sucrose		4.5 g. sucrose		5.5 g. sucrose	
	Proof time	Vol.	Proof time	Vol.	Proof time	Vol.	Proof time	Vol.
	min.	c.c.	min.	c.c.	min.	c.c.	min.	c.c.
1	69	575	62	580	58	575	59	580
2	51	590	51	600	48	570	45	590
3	67	560	64	555	59	570	57	580
4	62	590	65	570	60	590	60	605
5	62	590	62	585	60	580	54	580
6	43	560	49	550	50	560	50	550

The data in Tables II and III do not suggest the necessity for a high degree of precision in the adjustment of sugar levels in experimental baking. Varying the quantity of added sugar over a range of from 2.5 to 5.5 g. for the most part produced no significant variations in loaf properties other than crust color. Variations in responses to different sugar levels were surprisingly small whether proofed to constant height or to constant time, with perhaps slightly less variation when proofed to height.

The nature of the volume response to the two methods of proofing was not the same for all flours. Thus flour No. 1 gave significantly

larger volumes when proofed to height than when proofed to time, as did flour No. 3. With flour No. 2, however, the effect was distinctly the opposite. The range of variation from one flour to another was less when proofed to height than when proofed to time. The flours show a considerable range of variation as to time required to proof to standard height, regardless of sugar level. This probably was due at least in part to variations in Factor M.

### Varying Sugar Levels with Shortening

The experiments represented by the data in Tables II and III were repeated, using in all bakes 2% of shortening (Crisco) in the dough formula. This study was deemed advisable in view of the fact that shortening is frequently observed to be an important factor influencing flour responses and flour behavior. The influence of shortening on the loaf-volume responses to additions of varying quantities of sucrose is shown in Tables IV and V.

TABLE IV

LOAF VOLUMES FOR VARIOUS SUGAR LEVELS WHEN PROOFING TO STANDARD TIME (55 MINUTES) WITH 2% OF SHORTENING

Flour	2.5 g. sucrose Loaf vol.	3.5 g. sucrose Loaf vol.	4.5 g. sucrose Loaf vol.	5.5 g. sucrose Loaf vol.
	<i>c.c.</i>	<i>c.c.</i>	<i>c.c.</i>	<i>c.c.</i>
1	575	565	630	610
2	645	650	650	710
3	560	570	605	605
4	600	605	660	670
5	595	605	630	635
6	670	670	640	640

TABLE V

LOAF VOLUME FOR VARIOUS SUGAR LEVELS WHEN PROOFING TO STANDARD HEIGHT (9.5 CM.) WITH 2% OF SHORTENING

Flour	2.5 g. sucrose Loaf vol.	3.5 g. sucrose Loaf vol.	4.5 g. sucrose Loaf vol.	5.5 g. sucrose Loaf vol.
	<i>c.c.</i>	<i>c.c.</i>	<i>c.c.</i>	<i>c.c.</i>
1	595	615	635	635
2	655	635	655	670
3	580	605	580	620
4	660	670	685	695
5	640	630	645	660
6	630	630	650	660

The data in Tables IV and V indicate that the use of shortening tended to increase loaf volumes at all sugar levels. It is also apparent that the use of shortening favored a marked tendency toward positive volume response to *higher sugar levels* when the doughs were proofed

to standard time. This was not evident for Nos. 2 and 6, but for 1, 3, 4, and 5, the volumes were significantly higher at the two higher sugar levels than at the two lower ones.

Proofing to standard *height*, however, tended to equalize loaf volumes over the entire range of sugar levels.

Shortening is used in commercial practice. Since it is a factor influencing flour responses, it should perhaps be used in experimental baking.

The above experiments support the contention of Sandstedt and Blish (1938) that the safest procedure is to use a liberal quantity of sugar and proof to standard *height*. This should reduce the chances of unjust discrimination against flours that tend to proof slowly under conditions of the straight dough method, but which would not be of any serious disadvantage in commercial baking where the sponge method predominates. The possibilities in this connection are photographically illustrated in Figure 1.



Fig. 1. Overcoming the effect of different sugar levels by proofing to height instead of to time.

The loaves in Figure 1 were all baked from the same flour. Loaves O and P were baked with  $2\frac{1}{2}$  and 5 g. of sugar, respectively, and proofed to the same height. Loaves Q and R were also baked with  $2\frac{1}{2}$  and 5 g. of sugar, respectively, but proofed to the same *time*. This method of proofing unjustly penalized Q both in volume and in oven-spring, as compared with O, which was proofed to *height*. The higher sugar level in R practically offsets the slowness of proof. When proofed to height, however, there is no particular advantage in favor of the higher sugar level.

These considerations are further illustrated photographically in Figure 2. Loaves F, G, and H were baked from three different flours with  $2\frac{1}{2}$  g. of sugar, and proofed for 55 minutes. F is a slow proofing flour (proofs to 9.0 cm. in 55 minutes), while G and H are rapid proofers (proof to 11.5 cm. in 55 minutes). I, J, and K are the same flours, baked as before but proofed to the same height (9.5 cm.).

Flour F, a slow proofer, gave a much better oven-spring when proofed to height (see I) than when proofed to the standard time. As proofed to time (F) it might be considered as "underproofed." Flour H, a rapid proofer, when proofed to time, showed little oven-spring and was obviously "overproofed." This, also, was corrected by proofing to height (9.5 cm.) as shown in loaf K. A similar condition exists in the case of the flour represented by loaves G and J.

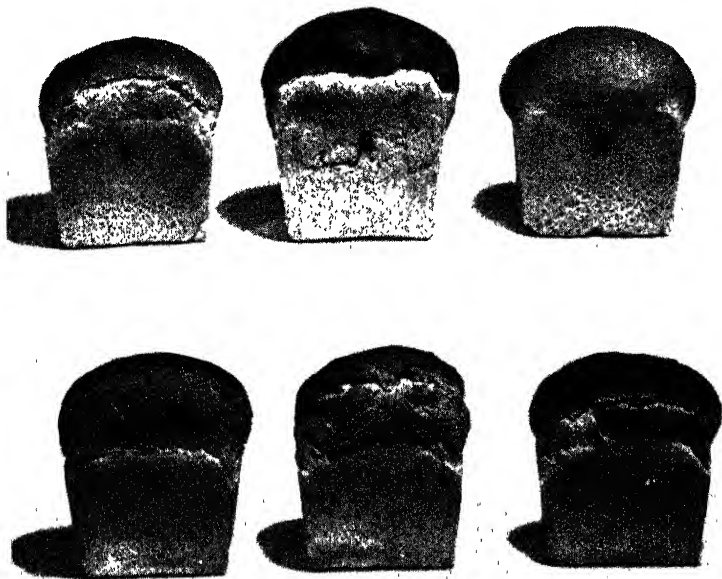


Fig. 2. Proofing to standard time vs. standard height.

From the experiments herein reported it is possible to conclude that in most experimental baking the safest all-around procedure is to use shortening, to adopt a sugar level somewhere in the range afforded by the addition of 4.5 to 5 g. of sugar for commercial flours of average gassing power, and to proof to constant height rather than to constant time. A height of 9.5 cm. has been used preferentially in these studies, but some other height might, as a result of additional study, be established as more suitable. This will perhaps depend upon the type of pan used or eventually adopted.

#### Summary and Conclusions

Six commercial bakers' flours of varying types, but all having at least *average* gassing powers, were experimentally baked at different

sugar levels. The effect of varying sugar levels was observed with reference to proofing to standard time as against proofing to constant height. In these connections the influence of shortening was also noted.

Aside from crust color, the effects on loaf properties produced by variations in added sucrose over a range of 2.5 to 5.5 g. were surprisingly unimportant, when shortening was omitted from the dough formula. With shortening, however, a positive volume response was effected in the higher sugar levels when doughs were proofed to standard time, but this effect was minimized when the proofing was to constant height.

In establishing a rational basis for interpretation of experimental baking tests, the use of shortening, of relatively high sugar levels, and proofing of the doughs to constant height are features which appear to offer the safest all-round procedure.

A standard or constant sugar level does not insure a constant rate of gas production.

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## EFFECT OF ADDING ALPHA- AND BETA-AMYLASES TO DOUGHS<sup>1</sup>

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(Received for publication June 22, 1938)

Numerous investigations of the importance of the diastatic enzymes to fermentation and gas production in doughs have been conducted, and Landis and Frey (1936) have discussed these thoroughly in an excellent historical review. The majority of the studies have involved malt extracts, malted wheat flours, and commercial malt diastase preparations. Such preparations contain both alpha- and beta-amylases in various proportions. Alpha-amylase, the dextrinizing enzyme, is generally considered most important in relation to diastatic activity. Recently the work of Blish, Sandstedt, and Mecham (1937) indicates that a "raw starch factor" is always found

<sup>1</sup> Paper No. 1625, Journal Series, Minnesota Agricultural Experiment Station.

present in alpha-amylase preparations and is of importance in raw-starch hydrolysis. Beta-amylase, the saccharifying enzyme, is usually found in abundance in wheat flours. This enzyme attacks the labile

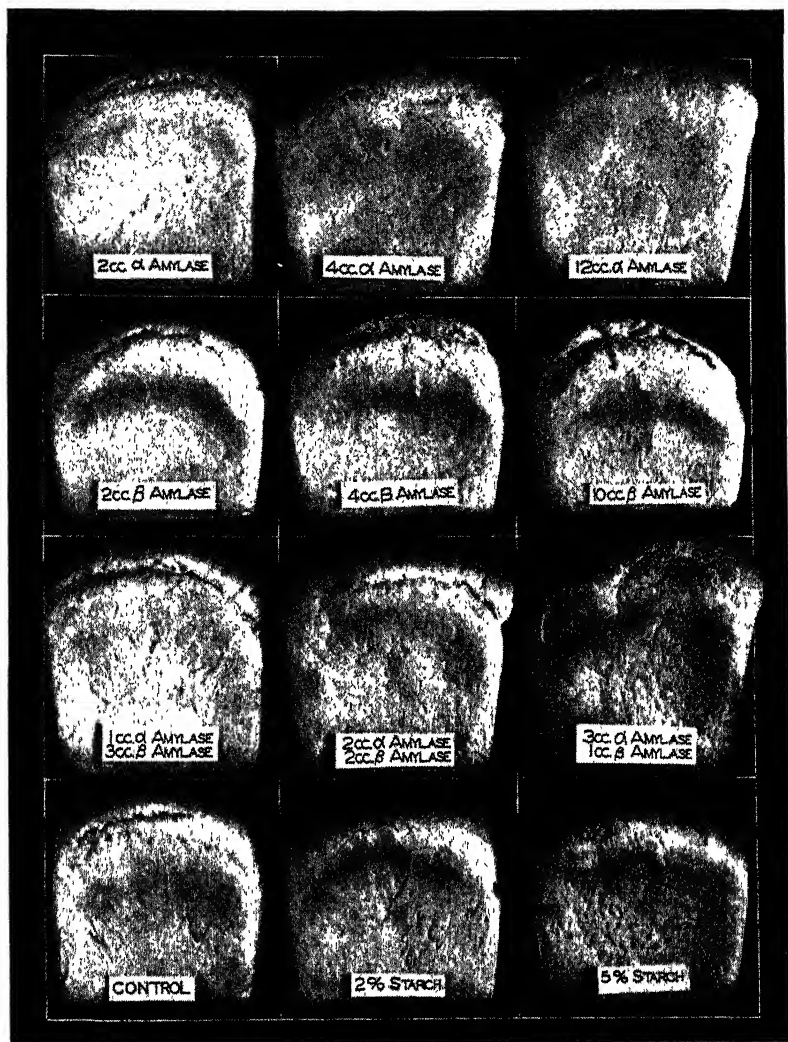


Fig. 1. Exterior of loaves made with various amounts and combinations of amylases and finely ground wheat starch. Fermentation time was three hours.

portions of the starch, and also the dextrins produced by alpha-amylase preparations. Thus the increase in diastatic activity upon overgrinding flours is mainly due to the action of beta-amylase on the fine fragments of broken-down starch granules.

The purpose of this work was to study the effect of alpha- and beta-amylases added to doughs, instead of diastase preparations with

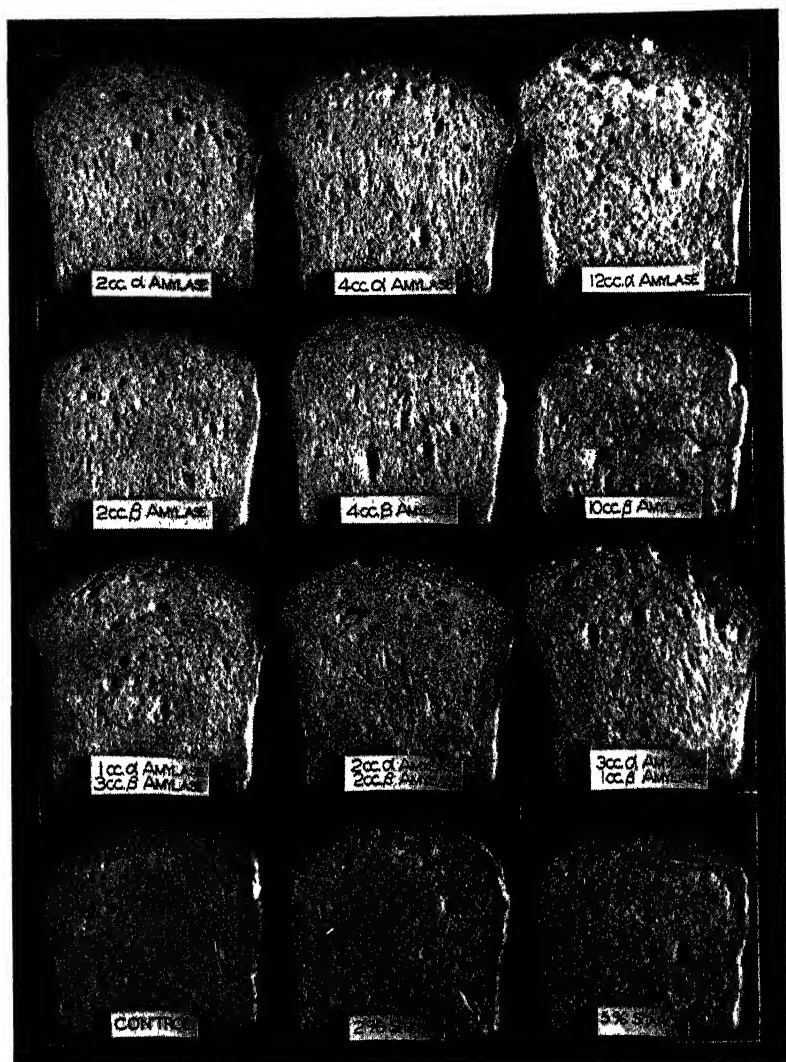


Fig. 2. Interior of loaves made with various amounts and combinations of amylases and finely ground wheat starch. Fermentation time was three hours.

appreciable amounts of both enzymes, and to obtain a pictorial record of the effect of these amylases on the bread when used in various amounts.

### Experimental

The alpha-amylase preparation was made by Ohlsson's (1926) technique. An extract from germinated wheat was heated to 70° for 15 minutes to inactivate most of the beta-amylase present.

The beta-amylase preparation was an extract from normal wheat which was acidified by HCl to pH 3.3 for 15 minutes at 0° (Ohlsson, 1926) and then brought to pH 6.0 by secondary sodium phosphate.

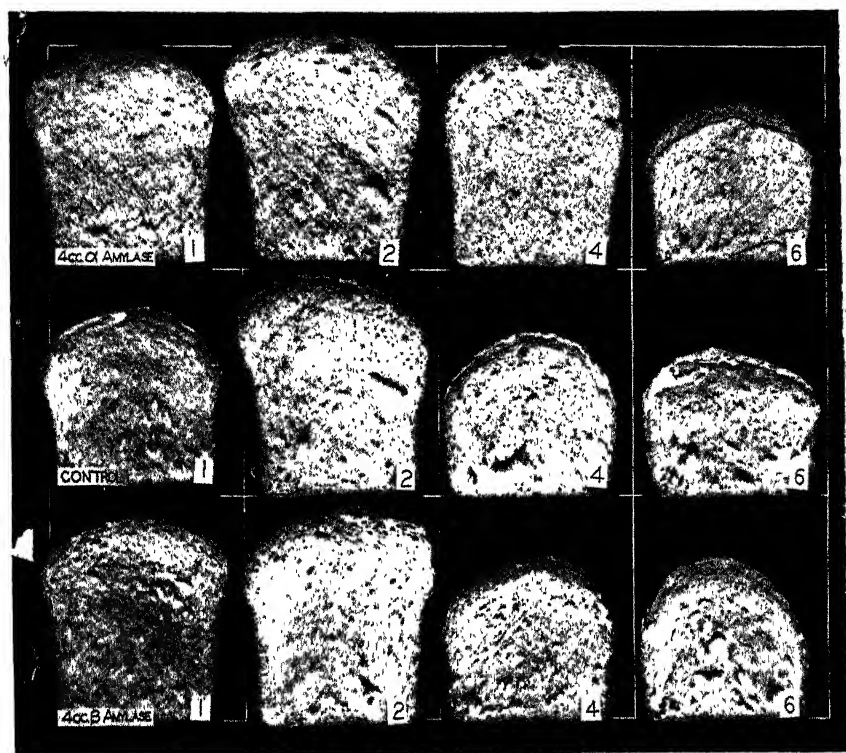


Fig. 3. Interior of bread made with 4 cc. of alpha- and beta-amylase in doughs with 1, 2, 4, and 6 hours of fermentation.

The extracts were kept at 0° until used. A small portion of the germinated wheat extract was used in precipitating alpha-amylase by adding alcohol to 60% concentration. Some of the normal-wheat extract was used also to precipitate beta-amylase at 80% concentration of alcohol. The precipitates were washed with absolute alcohol and dried under vacuum.

The alpha-amylase preparations exhibited high dextrinizing activity



on soluble starch, while the beta-amylase preparations showed no dextrinizing action on soluble starch but produced rapid saccharification.

The flour used for all baking tests was of medium strength, containing about 11% crude protein, and having a diastatic activity of 220 Rumsey units. The straight-dough procedure was used, and A. A. C. C. 100-gram loaves were baked. Each loaf contained the following ingredients:

100 g. flour	62% adsorption (includes enzyme solutions)
1% salt	no sugar
3% yeast	no shortening

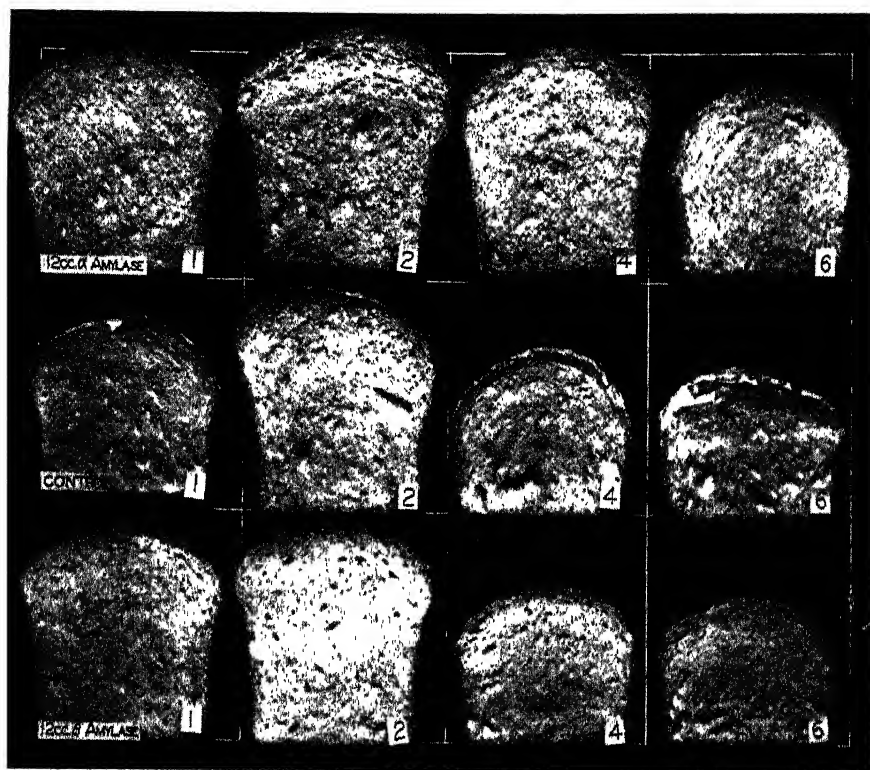


Fig. 4. Interior of bread made with 12 cc. alpha- and beta-amylase in doughs with 1, 2, 4, and 6 hours of fermentation.

The doughs were mixed for two minutes in the Hobart-Swanson mixer. The fermentation temperature was 30°. In one series of doughs the fermentation time was 3 hours, and in another series 1, 2, 4, 6, and 8 hours respectively. The pan proof was 55 minutes at 30°, and the loaves were baked for 25 minutes at 230°.

Finely pulverized wheat starch was added at 2% or 5% levels to certain doughs, replacing a like amount of flour. Thus the amount of substrate readily available for beta-amylase activity was increased in these instances. Sugar-free<sup>2</sup> doughs were used, since the presence of added sugar might obscure the effect of the amylases.

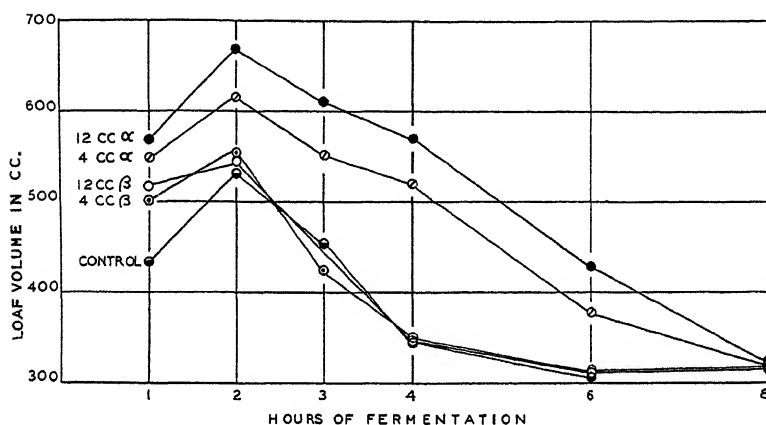


Fig. 5. The effect on loaf volume of the addition of alpha- and beta-amylase extracts to sugar-free doughs.

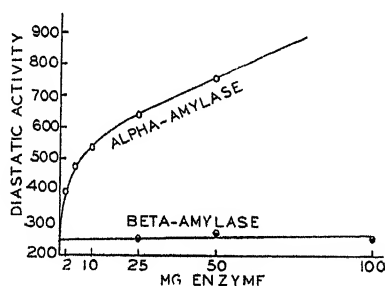


Fig. 6. Effect of the addition of alpha- and beta-amylase preparations upon the diastatic activity of flour, recorded in Rumsey units. The diastatic activity of the original flour was 245 R. units. Additions of enzymes are in terms of milligrams of the dry preparation per 5 g. flour.

Table I shows the effect on loaf volume and crust color of bread made with various additions, alone and in combination, of the enzyme extracts and also with the ground wheat starch added. Figures 1 and 2 show the exteriors and interiors, respectively, of the loaves produced.

Another series of doughs involved variations in fermentation time. The doughs accorded the longer fermentation of 2 to 8 hours were punched at every hour interval after mixing. Two dosages of enzymes were added, namely 4 c.c. and 12 c.c. of each enzyme extract.

<sup>2</sup> Meaning that no sugar was used in the formula.

TABLE I

BAKING TESTS WITH VARYING AMOUNTS AND COMBINATIONS OF  
AMYLASES AND GROUND STARCH

Three hours' fermentation time before panning—Figures 1 and 2 show the appearance  
of the loaves

Amount and kind of amylase solution or ground starch added			Loaf volume	Color of crust
Alpha-amylase	Beta-amylase			
<i>c.c.</i>	<i>c.c.</i>	<i>c.c.</i>		
0	(control)	0	454	++
2		0	543	++++
4		0	553	+++++
12		0	610	+++++
0		2	418	++
0		4	423	++
0		10	395	++
1		3	549	++++
2		2	550	++++
3		1	608	+++++
2% ground wheat starch			445	++++
5% ground wheat starch			450	+++++

++ = yellow.  
++++ = light-brown.  
+++++ = brown.

The results are recorded in Table II, in terms of loaf volume and crust color. Interiors of the corresponding loaves are depicted in Figures 3 and 4. Figure 5 records volume in c.c. of loaves baked from control doughs and from doughs that were fermented for varying intervals, and prepared with two proportions of alpha- and beta-amylase.

Amylases precipitated by alcohol in the manner already described were added in varying quantities to a flour similar to that used in these

TABLE II

THE EFFECTS OF DIFFERENT AMOUNTS OF ALPHA- AND BETA-AMYLASE WITH  
VARIOUS FERMENTATION TIMES UPON LOAF VOLUME AND CRUST COLOR  
(See Figures 3 and 4.)

Fermentation time	Alpha-amylase				Beta-amylase				Control	
	4 c.c.		12 c.c.		4 c.c.		12 c.c.		Loaf volume	Crust color
	Loaf volume	Crust color	Loaf volume	Crust color	Loaf volume	Crust color	Loaf volume	Crust color		
Hrs.	c.c.		c.c.		c.c.		c.c.		c.c.	
1	548	+++++	587	+++++	503	+++++	518	+++++	484	++++
2	615	+++++	668	+++++	553	++++	545	++++	532	++++
3	553	++++	610	++++	423	+++	—	—	454	+++
4	520	++++	572	++++	350	++	345	++	344	++
6	381	+++	429	+++	315	++	314	++	306	+
8	319	+	324	+	315	+	316	+	—	—

+ = pale-yellow.  
++ = yellow.  
+++ = light-brown.

++++ = brown.  
+++++ = dark-brown.

baking experiments. This flour had an original diastatic activity of 245 Rumsey units. The effects of these additions, here recorded in terms of milligrams of the dry enzyme preparation per 5 grams of flour, are indicated graphically in Figure 6, again in Rumsey units.

### Discussion

The data recorded in Table I and Figures 1 and 2 indicate that the addition of a small amount (4 c.c.) of alpha-amylase preparation to a normal, medium-strength flour dough improved the crust color, grain, and volume of the resulting loaves. The addition of a larger quantity of alpha-amylase (12 c.c.) produced a larger loaf but at the expense of grain and texture, which were inferior. The addition of beta-amylase in various amounts produced in all instances a poorer loaf of bread than the control. With increase in beta-amylase there was a decrease in loaf volume, paler crust color, poorer interior, and a greater tendency to give "shell top" loaves. In the various enzyme combinations used there was an improvement of the bread as alpha-amylase was increased up to 4 c.c. Beta-amylase appeared to be sufficient in the normal flour and the addition of beta-amylase had no improving effect. This is in accord with Andrews and Bailey (1934), who found that normal flours contain sufficient amounts of beta-amylase. The addition of finely pulverized wheat starch increased the diastatic activity of the dough as shown by the deeper crust colors, but the grain and texture were impaired, and there was no increase in loaf volume. The "break" was improved slightly. Alsberg and Griffing (1925) found that fine grinding of flours increases the diastatic activity but injures the baking quality.

Figures 3 and 4 show that the addition of small amounts of alpha-amylase (4 c.c.) improved the loaf volume considerably at the various fermentation times. Three times as much alpha-amylase (12 c.c.) increased the loaf volume slightly more, but inferior grain and texture resulted. Observations made are in agreement with those of Kozmin (1933), that flours made with the addition of too much sprouted wheat produce bread with defective qualities. The crumb loses its elasticity and dryness and appears damp and poorly baked. This is probably due to the dextrination of the starch by the enzymes beyond desirable limits, leaving insufficient unattacked starch for binding of the water in the dough during the baking process.

The addition of small or large amounts of beta-amylase did not improve the doughs, as shown in Figure 5, but an increased tendency to form "shell top" loaves was noticed after two hours' fermentation of the dough. When pulverized wheat starch was added the loaves

did not develop a shell top, although of about the same volume as loaves made with beta-amylase.

Figure 6 indicates that normal flours contain sufficient quantities of beta-amylase and that the addition of more beta-amylase does not result in a useful increase in the diastatic activity of the flour. Only small quantities of alpha-amylase or sprouted-wheat enzymes are necessary to produce a considerable increase in diastatic values of flours, since alpha-amylase, and its accompanying raw starch factor as explained by Blish and coworkers, readily attacks the starch in the flour to produce dextrin materials which are then saccharified by beta-amylase.

Care must be exercised in diastating doughs, and although large loaves are produced by increased diastatic activity, there is danger in overdiastating doughs with resulting inferior crumb, grain, and texture. This might be one reason for the sogginess of the crumb of some commercial bread found in certain markets.

### Summary

Alpha-amylase in small amounts added to sugar-free doughs improved the bread considerably, but larger amounts of alpha-amylase resulted in bread having inferior crumb, grain, and texture. Beta-amylase in small or large amounts did not improve the bread, and this enzyme appeared to be present in the flour in sufficient quantities.

Addition of finely pulverized wheat starch to the flour improved the crust color and "break" of the bread but had a detrimental effect on the grain and texture and produced no increase in volume.

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# FURTHER INVESTIGATIONS INTO THE NATURE OF THE ACTION OF BROMATES AND ASCORBIC ACID ON THE BAKING STRENGTH OF WHEAT FLOUR

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(Read at the Annual Meeting, May, 1938)

Considerable interest and discussion have been evoked by the author's papers (1935, 1935a) in which it was shown that oxidizing agents (especially potassium bromate), which act as flour improvers, inhibit the action of certain proteolytic enzymes; a theory was proposed that the pronounced improving effect of these agents on baking characteristics is to be ascribed to this inhibitory effect. Subsequently, several authors have published on this question and while several agree with the writer's theory, some others are not in accord, considering the author's interpretation of his experimental material (the correctness of which has not been contested) to be wrong. It is the purpose of the present paper to summarize the theory and its experimental basis, to examine critically a paper published by other investigators who interpret their experimental data as opposed to the writer's views, and to present additional supporting experimental evidence.

## The Theory and its Experimental Basis

The experiments published in the author's paper (1935) comprised the autolysis of flour suspensions containing small quantities of the various salts under study under fixed conditions and determining the quantity of nitrogen present in the aqueous extract. The presence of flour improvers such as potassium bromate and iodate during the autolysis of the flour-water suspensions depressed the nitrogen content of the extract, whereas potassium chlorate, which is not a flour improver, was without influence; moreover these depressions were greatly accentuated when the proteolytic activity of the flour being extracted was artificially increased by the addition of papain. Similarly, potassium bromate and iodate, but not potassium chlorate, inhibited the proteolytic activity of papain and the proteases of wheat flour, wheat germ, pineapple, and liver when acting on gelatin. Ascorbic acid, which is known to reduce the activity of plant proteinases of the papain group, was shown to be a good flour improver.

Another series of experiments published by Jørgensen (1935a) showed that the "bromate-depression," *i.e.*, the reduction in nitrogen solubility as a result of the presence of potassium bromate, is greater when the flour extractions are conducted in the presence of either compressed or dried baker's yeast; however, yeast was without influence on the bromate depression if the flour-proteinases were first destroyed by heat treatment of the flour. Nevertheless, the proteins of such heat-treated flour were not changed in such a way by heating that they could not readily be rendered soluble by papain and pineapple proteinase. Glutathione was found to exert an effect similar to that of yeast on the bromate depression; an aqueous extract of dried baker's yeast, free from yeast cells, gave a very strong nitro-prusside reaction showing that glutathione or similar compounds are given off from the dried yeast to the dough in bread-making.

On the basis of the experiments published in the first-mentioned paper the author (1935) concluded that oxidizing agents such as potassium bromate, which exert an improving effect on baking properties, owe their action to their inhibitory effect on the wheat-flour proteinases which, if allowed to act on the gluten at their normal rate, may diminish baking strength. While excessive proteolytic activity is detrimental, on the other hand, if it is too low, the gluten is "unripe." There is thus a certain optimum proteolytic activity which explains the fact that baking strength may be destroyed by an excessive dosage of oxidizing agents which in smaller amounts act as improvers. It was, however, stated to be an open question whether the very strong over-treatment which for example iodates may produce, is due exclusively to excessive inhibition of the flour proteinases.

On the basis of the experiments published in the last-mentioned paper the author (1935a) concluded that the proteolytic activity in doughs containing yeast is due to the *activation* of the flour proteinases by yeast and is *not* a result of the yeast itself secreting proteinases into the dough. In the instance of compressed yeast the mechanism of this activation is an open question, but with dried baker's yeast the activation is, in part at least, due to the yeast's giving off a proteinase activator, such as glutathione, to the dough. This stimulating effect of the yeast on the flour proteinases explains the well-known fact that potassium bromate has a greater effect on doughs to which yeast has been added.

### Corroboration of the Theory

Balls and Hale (1936) have shown that chlorine reduces flour proteolytic activity and, quite independently of the author, conclude that there is an optimum proteolytic activity for satisfactory ripening

of the gluten. Usually there is an excess and the beneficial effects of bleaching or natural aging are due to a diminution of proteolytic activity brought about by oxidation of the activator of the flour proteinase. They also conclude that similar effects are produced when the oxidant is added to the dough as a bread improver. The author's theory is also strongly supported by the experiments of Flohil (1936) and the investigations of Elion (1937) and Melville and Shattock (1938).

### The Work of Read and Haas

On the other hand, Read and Haas (1937), who repeated certain of the extraction studies of the author, secured results which they interpret as opposed to this theory and it is necessary critically to examine their experimental data. While these workers used a somewhat different technique, the flour-water ratio, namely 25 g. flour and 100 c.c. liquid, was the same as employed by the present author. Their extraction results for different quantities of potassium bromate, with normal wheat flour and also flour in which the proteolytic activity had been artificially increased by papain or pineapple juice, are presented in Table I.

TABLE I  
EXTRACTION TESTS WITH POTASSIUM BROMATE  
(From Read and Haas, Cereal Chem. 14: 753)

Extracted material	Mg. KBrO <sub>3</sub> added to extraction mixtures					
	0	0.25	0.5	1	50	100
	Mg. N in 20 c.c. of extract					
25 g. flour	14.3	14.3	14.3	14.3	13.6	13.5
25 g. flour + 50 mg. papain	86.5	86.2	85.1	81.0	29.1	23.4
25 g. flour + 10 mg. papain	43.0	43.0	42.1	—	16.7	15.3
25 g. flour + 5 drops of raw pineapple juice	46.3	46.2	43.6	43.2	16.9	—

On the basis of these results, Read and Haas (1937) conclude that the inhibitory effect of potassium bromate is evidenced only when it is added in amounts greatly in excess of those used in actual baking. The author, however, is of the opinion that their results actually confirm his theory. Certainly, in the instance of the tests where 50 mg. papain and pineapple juice were employed, 0.5 mg. potassium bromate (*i.e.*, 2 g. per 100 kg.) effected an appreciable reduction in nitrogen solubility and this quantity cannot be considered excessive. With wheat flour alone, 50 mg. of bromate definitely reduced the quantity of nitrogen dissolved, and while no effect is shown by the lesser quantities employed, the author believes that these smaller



amounts also produce a reduction but it is so slight as to escape detection under the conditions of such tests. It is believed that each increment of potassium bromate paralyzes a definite percentage of the total proteolytic activity and as this total is very small under the testing conditions employed, the relatively slight reduction brought about by the addition of small amounts of potassium bromate corresponds to an inappreciable decrease in nitrogen solubility. The correctness of this explanation is supported by the results of experiments presented later in this paper.

Read and Haas (1937) consider that reduction in the amount of dissolved nitrogen in the instance of normal flour cannot be ascribed to proteinase inhibition but must be explained otherwise, such as an electrolytic effect on the flour colloids. Moreover, they point out that this reduction is not evidenced until a 50-mg. dosage is used, an amount which is two hundred times greater than employed in bakery practice and they accordingly believe there is no connection between the reduction in dissolved nitrogen and the action of bromate on baking properties.

In reaching this conclusion, Read and Haas have failed to take two important points into consideration. First, the flour-water ratio in these extraction tests is 1 : 4, whereas in a fermenting dough it is approximately 1 : 0.6; consequently, in such tests the bromate is acting in a concentration only about one-seventh of that in fermenting doughs containing the same quantity of bromate. For this reason alone, larger amounts would be required to produce corresponding effects. Secondly, in a dough, *yeast* is present which activates the flour proteinases; hence the amounts of bromate necessary to show a reduction in nitrogen solubility in extraction tests without yeast cannot be directly compared with the quantities employed in bread doughs.

#### Extraction Tests with Potassium Bromate

That the presence of yeast greatly reduces the quantity of bromate required to reveal a reduction in nitrogen solubility is proved by the following experiments. Twenty-five grams of flour, commercially milled in Denmark from Manitoba No. 1 Northern wheat and containing 15.1% moisture, 2.45% nitrogen, and 0.58% ash, was extracted for 2 hours at 35° C. with an extraction mixture consisting of 75 c.c. water, 25 c.c. Sorensen phosphate mixture (pH 5.8), plus additions of potassium bromate. In the tests involving yeast, 0.5 g. ordinary baker's yeast (De Danske Spritfabrikker, Ltd., Copenhagen) was employed.

The extraction technique, which in such tests must be *well-defined*, was as follows: the extractions were carried out in 130 c.c. rubber-

stoppered centrifuge bottles; a large electrically-heated and regulated water thermostat was fitted with 12 holders mounted on a longitudinal axis and the extraction tests were carried out under water at a rotation speed of 25 r.p.m. In the tests involving yeast, the stoppers were tied in. After extraction, the bottles were centrifuged for 10 minutes in an Angle Separator (Stilles, Ltd., Stockholm) and the supernatant liquid filtered through S. and S. No. 589 filter paper; the extracts with yeast were filtered twice. The nitrogen in 20 c.c. of the filtrates was determined by the Kjeldahl method.

TABLE II  
EXTRACTION TESTS WITH POTASSIUM BROMATE  
(Author's Experiments)

Extracted material	Mg. KBrO <sub>3</sub> added to extraction mixtures					
	0	5	10	25	50	100
	Mg. N in 20 c.c. of extract					
25 g. flour	16.2	16.2	15.9	15.7	15.6	15.5
25 g. flour + 0.5 g. baker's yeast	20.1	18.9	18.6	18.0	18.1	17.3

The results, recorded in Table II, show clearly that 25 mg. potassium bromate reduces the amount of dissolved nitrogen when flour alone is extracted but when yeast is added the proteolytic strength is so much increased that a larger reduction is obtained with only 5 mg.; obviously, even smaller amounts would produce a distinct effect. When it is considered that this quantity of bromate is acting only in approximately one-seventh of the concentration which exists in actual fermenting doughs, it is clear that "bromate depression" is evidenced by quantities corresponding to those used in baking practice; in the Scandinavian countries, 1 to 5 g. bromate is employed for 100 kilos of flour.

#### Extraction Tests with Ascorbic Acid

According to the author's theory, ascorbic acid acts as a bread improver, because it, like potassium bromate and potassium iodate, also inhibits proteolytic activity. Read and Haas (1937) have carried out extraction tests with ascorbic acid similar to those they conducted with potassium bromate; their results, reproduced in Table III, are seemingly contrary to the writer's theory, since ascorbic acid produced a slight increase in water-soluble nitrogen.

It may easily be shown, however, that these results cannot be used as an argument against the theory. It is noteworthy that, while Read and Haas (1937) point out that 100 mg. potassium bromate is

TABLE III  
EXTRACTION TESTS WITH ASCORBIC ACID  
(From Read and Haas, Cereal Chem. 14: 753)

Extracted material	Mg. N in 20 c.c. of extract
25 g. flour	14.1
25 g. flour + 100 mg. ascorbic acid	14.2

four hundred times greater than the quantity employed in baking, this is the very (and only) amount of ascorbic acid which they used in their ascorbic-acid extraction tests; yet in practice this chemical is used in similar amounts to potassium bromate, namely, 1 to 2 g. per 100 kilos of flour.

To examine further the behavior of ascorbic acid, the author carried out a series of extraction tests with increasing amounts of ascorbic acid, using the same flour and technique as described for the bromate tests. In these experiments, pH was determined and in certain of the tests sodium hydroxide was added to prevent a change in pH through the addition of ascorbic acid. The results are summarized in Table IV.

TABLE IV  
EXTRACTION TESTS WITH ASCORBIC ACID

Series	Material extracted	Extract	Mg. ascorbic acid added to extraction mixtures					
			0	5	10	25	50	100
1.	Flour 25 g.	N in 20 c.c. (mg.) pH <sup>1</sup>	16.2 6.00	15.6 5.97	15.6 5.96	15.7 5.90	16.0 5.84	17.6 5.60
2.	Flour <sup>2</sup> 25 g.	N in 20 c.c. (mg.) pH <sup>1</sup>	16.2 6.01	15.6 6.00	15.5 6.01	15.5 6.00	15.7 6.02	15.8 6.01
3.	Flour 25 g. plus baker's yeast, 0.5 g. <sup>2</sup>	N in 20 c.c. (mg.)	20.1	18.4	18.6	18.4	18.7	

<sup>1</sup> Determined at 22° C. by the glass electrode.

<sup>2</sup> 0.038 c.c. N/10 NaOH was added per mg. ascorbic acid.

From the first series of experiments, it will be noted that, in agreement with Read and Haas, the addition of 100 mg. ascorbic acid increases the water-soluble nitrogen; however, the smaller amounts do not produce such an increase and, in fact, there is a distinct indication that the 5, 10, and 25 mg. additions result in a decrease. The pH values show clearly that the use of 100 mg. ascorbic acid lowers the pH of the extract from 6.00 to 5.60 and this appreciable decrease in pH

is the reason for the higher nitrogen content of this particular extract. A suitable addition of hydrochloric acid will, in itself, increase the water-soluble nitrogen and, consequently, this is a simple pH phenomenon and not a specific effect of ascorbic acid. In practice, 1 to 2 g. ascorbic acid per 100 kilos are used and these amounts do not produce a measurable decrease in pH. For example, a dough consisting of 280 g. flour, 4 g. salt, and 160 c.c. water was found by direct measurement with the glass electrode to be pH 5.95 (23° C.) and a similar dough containing 5.6 mg. ascorbic acid (equivalent to 2 g. per 100 kilos) showed precisely the same value.

In order properly to study the influence of ascorbic acid on proteinase activity by extraction tests, it is obviously necessary to eliminate the pH effect. This has been done in the second and third series of experiments recorded in Table IV, and under these conditions, it is seen that ascorbic acid reduces the water-soluble nitrogen, the reduction being much more pronounced when yeast is also present. These extraction tests thus afford excellent support for the author's theory that ascorbic acid acts as a baking-strength improver because it inhibits the flour proteinases.

The question why ascorbic acid, which is a reducing agent, produces an effect similar to that of potassium bromate, an oxidizing agent, remains to be answered. In a series of patent applications,<sup>1</sup> the author has advanced the view that ascorbic acid may possibly act as an oxygen carrier, absorbing oxygen from the atmosphere and the oxidized acid then imparting the oxygen to the flour, thereby inactivating the flour proteinases. This hypothesis has recently been supported experimentally by Melville and Shattock (1938).

### The Nature of Wheat Flour Proteinases

As is well known (see Grassman and Schneider, 1936), proteinase enzymes may be classified into three main groups: (1) the pepsin group, optimum pH 2, approximately; (2) the papain-cathepsin group, optimum pH 4 to 7; and (3) the trypsin group, optimum pH 8, approximately.

Balls and Hale (1935, 1936) maintain that the flour proteinases active in the baking process belong to the papain group. The author's investigations (1935) show an extraordinarily close analogy between papain and the wheat proteinases; Flohil (1936) also concludes that the wheat germ proteinases are of papain character. However, Read and Haas (1937) express doubt as to the correctness of these views because they interpreted their extraction tests with bromate as opposed to the

<sup>1</sup> U. S. Patent Application Serial No. 36039, filed Aug. 13, 1935. Corresponding New Zealand Patent Application No. 74836, filed Aug. 26, 1935, and open for public inspection from April 29, 1936.

proteinase inhibition theory—an interpretation which the author considers to be incorrect in the light of the additional experimental evidence presented as to the stimulating effect of yeast on the flour proteinases and also because of the differences in relative concentration of bromate in the aqueous suspensions and actual doughs.

There is good reason to class the wheat proteinases in the papain group. Falk and Winslow's work (1918), cited by Read and Haas, indicates that enzymes of the trypsin group are of little importance in baking-strength problems. These investigators studied the influence of potassium bromate on Merck's, Fairchild Brothers', and Foster's "Trypsin" and on Merck's "Pancreatin" (a trypsin preparation) when acting on casein as a substrate. They found that, particularly in low concentrations (1 part  $\text{KBrO}_3$  per 100,000 parts liquid), the activity of trypsin was increased by the bromate, whereas the activity of pancreatin was slightly inhibited at a bromate concentration of 1:10,000 and stimulated in lower concentrations (1:200,000). Since "Pancreatin" is also a trypsin preparation, it is curious that the results differ; however, careful examination of the work of Falk and Winslow indicates that their experimental technique did not permit of good agreement between parallel tests and it is felt that their conclusions on the relative effect of bromate on trypsin and pancreatin activity are hardly justified. If these workers had included papain in their studies, the strong inhibitory action of bromates on its activity would have been established and the fundamental nature of bromate action in relation to baking would have been cleared up many years ago. That the inhibiting action of bromate on papain, when acting on casein as a substrate, is very strong is shown in Table V, reproduced from a recent paper by the author (1938).

TABLE V  
DIGESTION OF CASEIN BY PAPAIN IN PRESENCE OF VARIOUS HALOGEN  
SALTS<sup>1</sup>

Halogen salt	Nitrogen rendered soluble per 20 c.c. extract
	mg.
KCl	16
KBr	16
KI	16
$\text{KClO}_3$	16
$\text{KBrO}_3$	3.9
$\text{KIO}_3$	3.5

<sup>1</sup> In these tests, 4 g. casein and 0.08 g. Witte's papain were extracted with 50 c.c. acetate buffer (pH 4.62) plus 50 c.c. 0.004 M solution of the specified halogen salts for 2 hours at 35° C.

### Summary

The author's theory that potassium bromate and ascorbic acid act as baking-strength improvers through inhibition of the flour proteinases is summarized and the work of Read and Haas, who interpret their experimental data as opposed to this theory, critically examined.

It has been pointed out that, in interpreting the results of their extraction tests, these workers did not take into consideration the stimulating effect of yeast on the flour proteinases or the fact that larger quantities of potassium bromate would be required in extraction tests than in doughs to produce a corresponding reduction in nitrogen solubility because of the much wider flour-water ratio. Experiments are reported which show that, when due consideration is given to these points, dosages of bromate, corresponding to those used in baking, reduce the solubility of the flour nitrogen.

It is also proved that the increase in water-soluble nitrogen reported by Read and Haas through the use of an excessive amount of ascorbic acid, is due to a reduction in pH; when this pH effect is eliminated, ascorbic acid reduces nitrogen solubility in all dosages employed. The author's theory is thereby confirmed.

Evidence strongly points to the wheat proteinases as belonging to the papain group of proteinases. Potassium bromate and iodate strongly inhibit the activity of papain on casein.

### Acknowledgments

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## STUDIES ON THE BAKING QUALITY OF FLOUR AS AFFECTED BY CERTAIN ENZYME ACTIONS. VI. FURTHER STUDIES RELATING TO THE ACTIVATION AND INHIBITION OF FLOUR PROTEINASE<sup>1</sup>

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(Read at the Annual Meeting, May 1938)

In previous papers dealing with the baking quality of flour as influenced by certain enzyme actions, we have considered the following angles of the problem: (1) the beneficial effects of proteolytic activity on the baking behavior of flours which develop "bucky" doughs (1934); (2) the separation of proteinase from the amylases; baking tests designed to measure the effects of these enzymic factors on "bucky" doughs, and the liquefying power of diastatic agents (1936); (3) purification of amylase, and the relative potency of the several enzymic fractions (1937); and (4), (5) the response of some proteinases to oxidizing and reducing agents with reference to activation and inactivation (1937 and 1938).

The results to be recorded in the present paper are essentially an elaboration of the two latter studies, conducted for the purpose of securing additional experimental information relative to the activation and inactivation of the proteinase contained in flour and malt products.

<sup>1</sup> Subsequent to the preparation of this manuscript for the Cincinnati convention of the American Association of Cereal Chemists, H. Jørgensen (*in absentia*) offered a paper at the same meeting in which he took issue with some previously published findings of Read and Haas. Although the latter have not yet had an opportunity to read Jørgensen's paper in full, they consider that the results here presented constitute further evidence in support of their views as previously published.

### Brief Review of Literature

Mueller (1936) reported valuable studies concerned with the evaluation of dough quality by physical means. Experiments designed to show the effect of chemical treatment on the aging and extensibility of flour doughs failed to support Jørgensen's theory, that the benefits derived from bromate result entirely from proteinase inhibition. Physical studies of this particular nature, apparently definitely related to the bromate problem, give rise to questions other than the concept of inhibition.

In a paper concerned with the baking quality of flour doughs in relation to softening of the dough with age, Halton (1938) has pointed out that this phenomenon may be attributed to syneresis just as satisfactorily as it may be credited to enzyme action, as stated by Jørgensen (1936).

Sullivan, Howe, and Schmalz (1936) have cautioned against too narrow a view regarding the activation of proteolytic enzymes by substances containing the sulfhydryl group, because many compounds, inorganic as well as organic, can materially influence the colloidal behavior of the gluten proteins by modifying the oxidation-reduction system.

Hills and Bailey (1938) found that papain digestion increased the  $\beta$ -amylase activity of ungerminated barley approximately 100%, due to proteolytic release of  $\beta$ -amylase associated with water-soluble material.

Elion and Elion (1932) patented the process of increasing the saccharogenic ( $\beta$ -amylase) activity of flour appreciably by applying persulphate of potassium or ammonium in amounts varying from 0.25 to 0.02% by weight of flour. This is interesting in connection with the results of Hills and Bailey just cited.

Swanson (1937), using the recording dough mixer, evaluated graphically the condition produced in doughs by several treatments. Certain of Swanson's findings are at variance with those of Jørgensen (1936), namely the presence of significant quantities of free activating factors in a supercentrifuged aqueous extract of macerated yeast. The proteinase activity of malt wheat flour, if of any significance, was too slight to be detected, but the malt flour acted to make the dough more pliable. Proteinase activity (pepsin) was not inhibited by bromate.

Freilich and Frey (1937) reported that the incorporation of oxygen into bread doughs inhibited the effect of papain, although indirectly, since the inhibition is directly proportional to the amount of flour treated with oxygen. Treating papain alone with oxygen did not reduce its potency.



In connection with studies dealing with the action of ascorbic acid as a bread improver, Melville and Shattock (1938) have stated that dehydro-ascorbic acid is equivalent to bromate on a weight-for-weight basis, and is about four times more potent than is ascorbic acid on flours requiring 0.004% of bromate, an excessive dosage for flours in

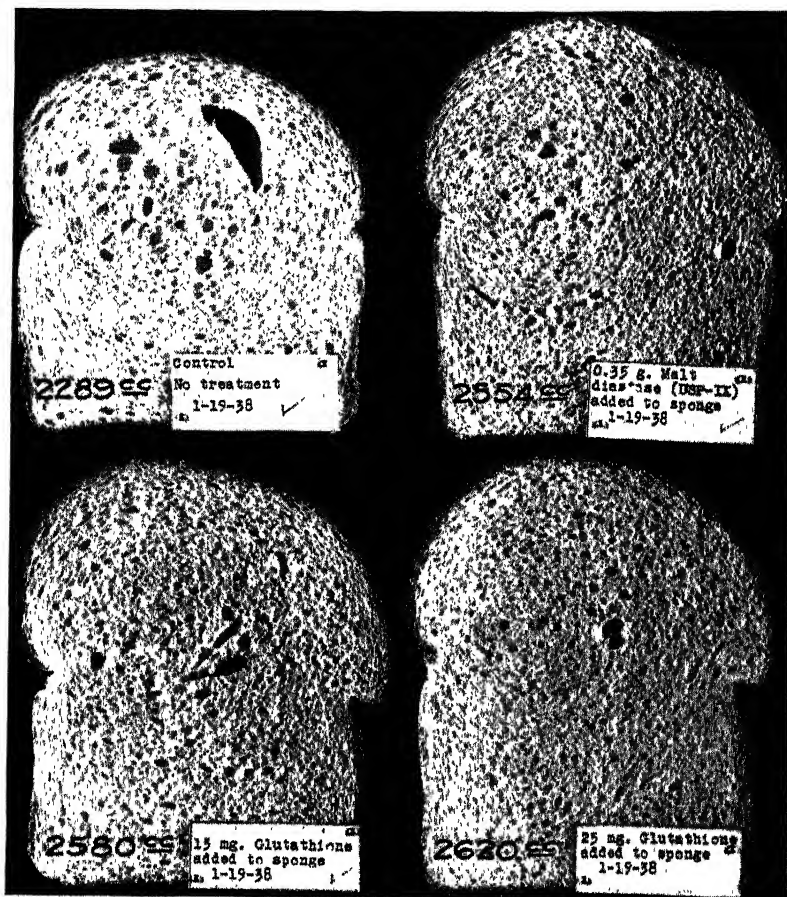


Fig. 1. Baking results with flour No. 167880, showing effect on crumb structure and loaf volume produced by suitable amounts of glutathione, as compared to control (no treatment) and a 0.35-g. application of Merck's diastase of malt (U. S. P. IX), a medicinal preparation which is highly proteolytic. Additions were made to the sponge.

general. On the basis of data secured, they hold to the opinion that ascorbic acid *per se* is inactive in relation to such flours, and state that flour contains a mechanism whereby the oxidation of ascorbic acid to dehydro-ascorbic is brought about by an enzyme commonly called "ascorbic acid oxidase," a factor which would probably exist in variable amounts in flours, and thereby might explain the discrepancies

which appeared in some of their experiments conducted on a range of flours.

Hopkins and Morgan (1936) made a quantitative investigation of the system glutathione—ascorbic acid—enzyme. They found that so long as glutathione remained in the system it completely prevented

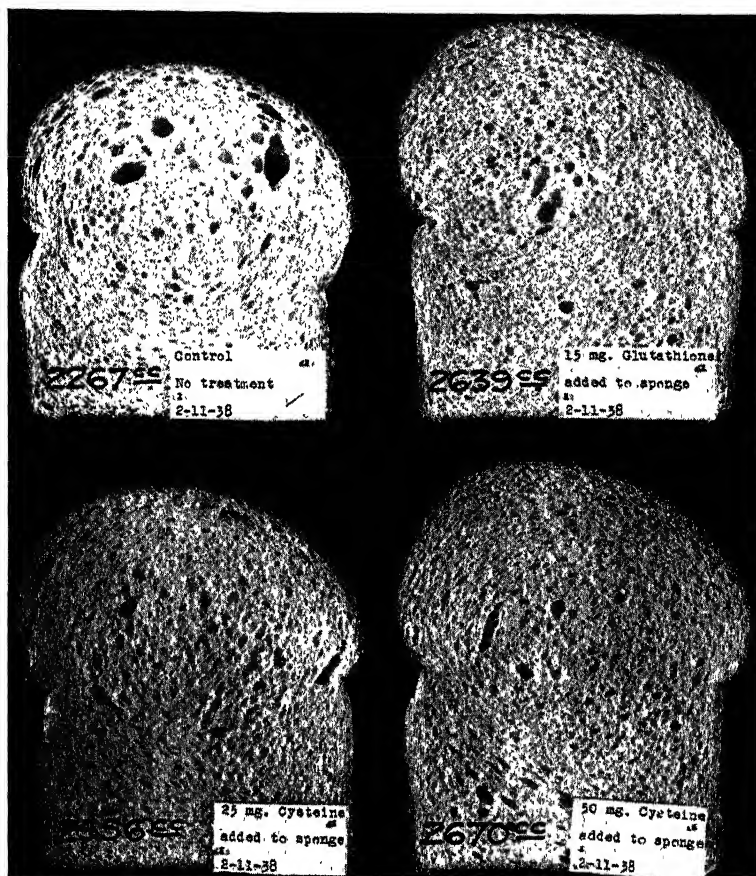


Fig. 2. Baking tests with flour No. 167880, showing effect on crumb structure and loaf volume produced by suitable quantities of glutathione and cysteine when added to the sponge.

the oxidation of ascorbic acid, but was itself oxidized. Only when glutathione had practically disappeared from the system did the oxidation of ascorbic acid begin. Glutathione also completely protected ascorbic acid from oxidation by copper catalysis.

Stotz, Carter, and King (1937) have criticized the use of "ascorbic acid oxidase," "vitamin C oxidase," and "hexuronic acid oxidase," as

interchangeable terms for a supposedly enzymic factor which acts in a specific manner to catalyze the aerobic oxidation of vitamin C. They suggest that some copper complex may perform the catalytic role.

Halton and Scott Blair (1937) have considered certain physical properties of flour doughs in relation to their bread-making qualities.

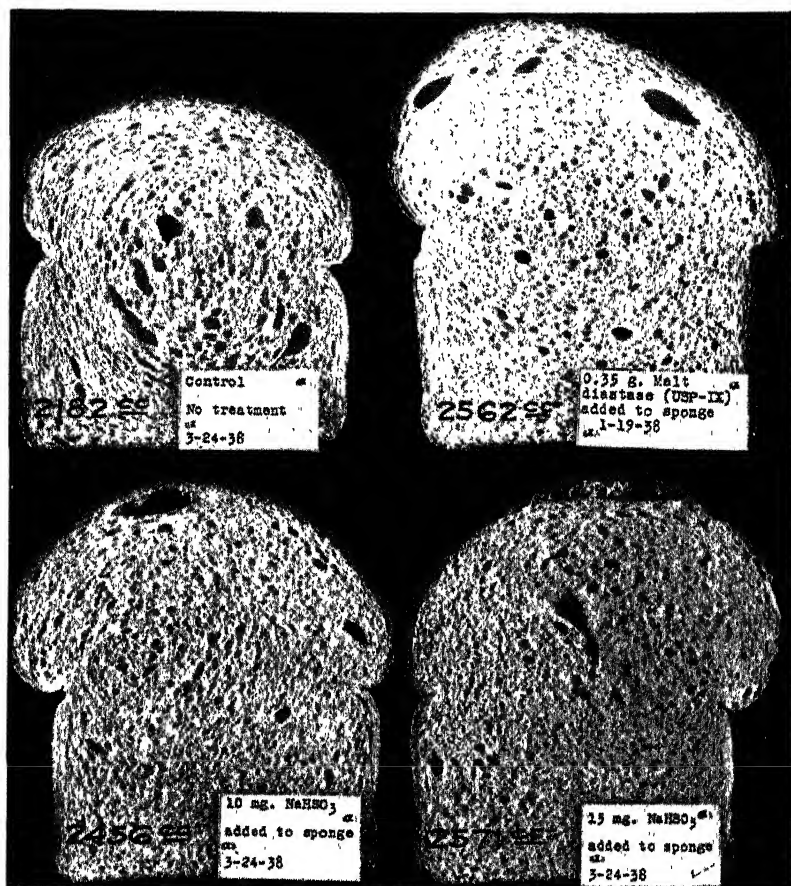


Fig. 3. Baking results with flour No. 167880, showing effect on crumb structure and loaf volume produced by suitable quantities of sodium bisulphite when added to the sponge. Compare to control; also the customary dosage of Merck's diastase of malt—U. S. P. IX, which we have applied to flours giving rise to "bucky" doughs. This product is highly proteolytic.

They have pictured, rather aptly, the mechanism of dough behavior during fermentation, wherein the gluten of flour dough consists of protein chains, which, acting like coiled springs, are responsible for the elastic behavior of the dough. Reference is made to this publication because it is probable that investigations of this type offer definite

angles of attack for determining the role (multiple or otherwise) played by bromate in baking processes.

### Experimental

*Flour.*—The flour (No. 167880) used for the baking tests was milled from 50-lb. test wheat grown under adverse dry-weather conditions. Soon after cutting, excessive rainfall caused some sprouting in certain areas. Apparently, this accounts for its abnormally high saccharogenic activity of 642 (Blish-Sandstedt units), and a gassing power of 2036 c.c. (per 100 g. flour) as measured on the Werner Gasometer. The flour had been rather heavily Agene bleached, and whether its "bucky" character should be credited chiefly to overbleaching or to a combination of causes is uncertain.

*Baking tests.*—Using sponge doughs, numerous baking tests were made to determine the response of this flour to varying amounts of cysteine, glutathione, and sodium bisulphite when incorporated in the sponge. Results of these tests are illustrated in Figures 1, 2 and 3. Our interest in making these tests centered in the possible differences in the action of these agents on the gluten as determined by "feel" and "appearance" of the dough, and also the proper dosage required to produce good loaves from a flour giving rise to "bucky" doughs.

*Gelatin liquefaction.*—As in previous studies, 25 c.c. of a 5% gelatin solution was used for evaluating the relative amount of liquefaction under specified conditions. The data contained in Tables I and II represent the conclusions reached from a study of a large number of tests, which, if tabulated separately, would require numerous tables. Relative viscosities, measured in terms of the time factor involved to deliver a fixed volume from a 100 c.c. specified pipette, gave similar results. It is realized that results obtained on a gelatin substrate might differ from those obtainable on gluten. A 5% solution of the gelatin used throughout our studies showed a pH range of from 5.4 to 5.7. The gelatin was an imported product always purchased from the same source. The pH of ordinary gelatins may vary widely.

*Malt flour (wheat) XXII.*—The wheat from which this flour was prepared in the laboratory was germinated for eight days at around 52° F. The average acrospire length was approximately 0.75 inch. After drying and removal of vegetative growth, the grain was ground and sieved. The malt flour, as used, consisted of the portion passing through a standard 80-mesh sieve. The saccharogenic index of this malt was 825, an unusually high index value. Ordinary malt flours generally range from 200 to 250. The proteolytic power of the specially prepared malt was correspondingly high, thereby making it

suitable material for gelatin studies. Commercial malt flours were not found to be very satisfactory because of their relatively weak proteolytic activity.

*Sodium acid sulphite.*—The incorporation of suitable quantities of sodium acid sulphite in the sponge mix produced essentially the same net results in conditioning the gluten as did glutathione and cysteine, but the behavior of this salt toward gluten appeared to be different. The “feel” and fibrous condition of the sponge was so dissimilar that it became immediately noticeable after a given period of fermentation. This salt has been referred to as an activator for papainases, but it will be observed from Table I that it greatly repressed the proteolytic activity of papain, bromelin and malt flour XXII.

*Glutathione.*—The glutathione was purchased from Eastman Kodak Co., but not prepared by them. We understand it is of German manufacture. Further information was unavailable except that it was considered to be essentially the reduced form.

TABLE I  
RESPONSE OF CERTAIN PROTEASES TO REDUCING AGENTS

Enzymic product	<i>Treatment applied</i>			
	5 and 10 mg. glutathione	5 and 10 mg. cysteine	5 and 10 mg. vitamin C	5 and 10 mg. NaHSO <sub>3</sub>
Merck's diastase U. S. P. IX <sup>1</sup>	Slight inhibition	Slight inhibition	Slight inhibition	Appreciable inhibition
Taka diastase	Slight inhibition	Slight inhibition	Slight inhibition	Appreciable inhibition
Papain	Marked activation	Marked activation	Marked inhibition	Marked inhibition
Bromelin (pineapple juice)	Marked activation	Marked activation	Marked inhibition	Marked inhibition
Trypsin	Slight inhibition	Slight inhibition	Slight inhibition	Slight inhibition
Powdered malt extract <sup>1</sup> (103° L.)	No activation	No activation	Appreciable inhibition	Without effect
Special malt flour (wheat) XXII	Marked activation	Marked activation	No inhibition	Marked inhibition
Malt syrup <sup>1</sup> (390° L.)	No activation	No activation	Slight inhibition	Slight inhibition

<sup>1</sup> It will be observed from this table that malt products which had been processed exhibited a different response from the wheat malt flour XXII.

TABLE II  
RESPONSE OF CERTAIN PROTEASES TO OXIDANTS

Enzymic product	<i>Treatment applied</i> <sup>1</sup>		
	5 and 10 mg. NaClO <sub>2</sub>	5 and 10 mg. KBrO <sub>3</sub>	KH(IO <sub>3</sub> ) <sub>2</sub> KIO <sub>4</sub> K <sub>2</sub> S <sub>2</sub> O <sub>8</sub> (Dosage: 1 c.c. N/10)
Merck's diastase U. S. P. IX	Slight inhibition	Very slight inhibition	Very slight inhibition
Taka diastase	Slight inhibition	No inhibition	Very slight inhibition
Papain	Marked inhibition	Marked inhibition	Marked inhibition
Bromelin (pineapple juice)	Marked inhibition	Marked inhibition	Marked inhibition
Trypsin	Slight inhibition	No inhibition	No appreciable effect
Powdered malt extract (103° L.)	Appreciable inhibition	Very slight inhibition	Inhibitive action slight
Special malt flour (wheat) XXII	Appreciable inhibition	Appreciable inhibition	Measurable inhibition
Malt syrup (390° L.)	Appreciable inhibition	Measurable inhibition	Measurable inhibition

<sup>1</sup> The quantities of oxidants listed in this table are very excessive in relation to the dosages of enzymic product required. Proper enzymic controls were run simultaneously. With reference to the four malt products, no apparently significant repressive action was detected when the potassium salts were applied in quantities suitable for baking practice.

### Discussion

The data recorded in Tables I and II show the response which followed the application of certain oxidizing and reducing agents to eight enzymic products, three of which are utilized in commercial baking. All have proteolytic activity. It will be observed from Table I that reducing agents, such as glutathione, cysteine, vitamin C, and sodium bisulphite, exert a varied influence. Papain, bromelin, and malt flour were activated by the presence of glutathione, while Merck's diastase of malt, powdered malt extract, malt syrup, trypsin, and taka diastase, were either slightly inhibited or failed to respond. The same was true for cysteine which in common with glutathione contains the sulfhydryl group. The other two reducing agents, vitamin C and NaHSO<sub>3</sub> exerted in every case an inhibitory action of varying degree. These statements are based on results obtained with a gelatin substrate. It should be noted that processed malt products, such as powdered malt extract, malt syrup, and Merck's diastase of

malt (U.S.P. IX) responded differently from wheat malt flour in its normal state.

Table II shows the reaction of the same proteolytically active agents toward certain oxidants. Here again we find that the response of papain and bromelin is far more pronounced than is that of the several malt products, together with trypsin and taka diastase. It is recognized that an application of 5 or 10 mg. (per 25 c.c. gelatin solution) is a very excessive dosage when considered in relation to the quantity of enzymic agent. However, in obtaining the more complete information on the basis of gelatin liquefaction, not recorded in Tables I and II, the dosages of chemical agents were scaled downwards from 5 mg. to as little as 0.05 mg.—the smaller applications falling within the range of baking practice. We wish to call attention particularly to the fact that dosages within the range of baking practice produced no apparently significant inhibition on malt products adapted to baking, as measured by the technique we have employed. With reference to gelatin splitting, the proteinase of normal malt flour was found to be far less responsive to inhibition by oxidants than was true for either commercially prepared papain, or for bromelin as it exists in the raw juice of the pineapple.

TABLE III  
EFFECT OF CERTAIN PRODUCTS ON THE pH OF GELATIN SOLUTION  
Additions were made to 25 c.c. of a 5% gelatin solution.

Treatment	pH
Gelatin control	5.68
5 mg. vitamin C	5.62
10 mg. vitamin C	5.52
20 mg. vitamin C	5.25
10 mg. cysteine hydrochloride	5.35
10 mg. glutathione	5.36
10 mg. NaHSO <sub>3</sub>	5.57
2 g. special malt syrup (390° L.)	5.52
4 g. dry malt	5.41
5 g. ordinary malt flour	5.55
2 g. wheat malt flour XXII	5.52

All pH determinations in this study were made by means of the glass electrode immediately after the additions of the different reagents.

In Table IV are recorded the results of some tests conducted with vitamin C for the purpose of comparing its behavior toward papain and malt flour XXII. These data are informative, but do not justify conclusions before subjecting certain points to further detailed study. It may be noted, however, that papain and malt flour responded differently under approximately identical conditions except for slight variations in pH.

TABLE IV  
COMPARATIVE EFFECT OF VITAMIN C ON WHEAT MALT FLOUR NO. XXII AND  
PAPAIN

Quantity enzymic agent added per flask		Vitamin C applications shown in milligrams						Enzymic control	Gelatin control	
		5	2	1	0.5	0.2	0.1			0.05
Section A, papain (8% aqueous extract)										
Consistency of gelatin after 20 hours' digestion at 30° C.										
0.3 c.c.	30°	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0°	Very firm	Fairly firm	Fairly mobile	Viscous liquid	Viscous liquid	Viscous liquid	Liquid	Liquid	Very firm
pH		5.34	5.40	5.44	5.48	5.51	5.51	5.53	5.53	5.58
Section B, wheat malt flour XXII										
Consistency of gelatin after 20 hours' digestion at 30° C.										
2 grams	30°	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0°	Very viscous liquid	Very soft gel	Soft gel	Soft gel	Soft gel	Soft gel	Soft gel	Soft gel	Very firm
pH		5.48	5.51	5.52	5.53	5.53	5.54	5.55	5.55	5.60
Consistency of gelatin after 24 hours' digestion at 35° C										
2 grams	30°	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0°	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very viscous liquid	Very firm
pH		5.38	5.43	5.46	5.47	5.48	5.48	5.50	5.52	5.56

### Summary

With the incorporation of suitable quantities of glutathione, cysteine, and sodium acid sulphite in the sponge, baking tests with flour No. 167880 indicated that the nature of the action of the inorganic reagent, in conditioning the gluten, was unlike that of either glutathione or cysteine. Our tests on gelatin have shown that sodium acid sulphite strongly inhibited the proteolytic activity of papain, bromelin, and malt flour. Under similar conditions glutathione and cysteine functioned as potent activators.

Tests made on gelatin have shown that the proteinase of wheat malt is far less responsive to inhibition by bromate than are papain and bromelin. When applied in quantities applicable to commercial baking practice, no significant repression of wheat proteinase was apparent.

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# SOYBEAN AMYLASE. I. THE CONCENTRATION AND CHARACTERIZATION OF SOYBEAN AMYLASE<sup>1</sup>

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Studies have been carried on in this laboratory on the preparation and properties of amylase concentrates from germinated wheat and rye by Naylor, Spencer, and House (1925) and Creighton and Naylor (1933), from germinated corn by Bulbrook (1928), and from germinated oats by Naylor and Dawson (1936). This paper reports the results of preliminary investigations dealing with the amylase of soybeans.

Orestano and Zummo (1930) report, after heat-treatment studies, that their results do not indicate the existence of two different amylases in soybeans, one causing liquefaction and the other saccharification of starch. Later studies by Artom and Orestano (1931) led to the equations for liquefaction and saccharification by soybean amylase. Orestano (1933) also reports that soybean amylase contains only one amylase, probably  $\beta$ -amylase. Results of investigations by Teller (1936) indicate the presence of two amylases in soybeans, but show definitely that soybean amylase is a better sugar-former than any other amylase studied with the exception of the enzyme of dry sweet potato. Bray (1935) offers the interesting suggestion that the amount of available amylase in soybeans does not appear to increase during germination, and that the enzyme is chiefly  $\beta$ -amylase.

Amylases are usually classified by Kuhn's (1925) mutarotation method, by methods involving measurement of the quantity of reducing sugars produced by the action of the enzyme on starch paste, or by methods based on the liquefaction of starch paste as measured by viscosimetric procedures. Dextrinogenic activity is commonly estimated by the Wohlgemuth iodine method. The terms  $\alpha$ - and  $\beta$ -amylase are thus defined by the specific method used. Of the several methods Kuhn's is preferable, but it does not provide a means of measuring small amounts of one type of amylase in the presence of an excess of the

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other. As used in this paper the terms  $\alpha$ - and  $\beta$ -amylase are defined as follows:

$\beta$ -amylase (saccharogenic)—the enzyme which hydrolyzes starch to  $\beta$ -maltose, thus giving a positive mutarotation by Kuhn's method.

$\alpha$ -amylase (amylolytic)—the enzyme which liquefies and dextrinizes starch paste to products which will not give the typical starch-iodine blue as measured by the modified Wohlgemuth method of Creighton and Naylor (1933).

### Experimental

*Preparation of enzyme suspension.*—Two-tenths gram samples of soybean, ground to pass a 40-mesh sieve, were weighed directly into an agate mortar and ground as fine as possible. A small amount of water, redistilled and ice-cold, was added and grinding continued until a thick paste was obtained. This was gradually diluted to a thin liquid, transferred to a 100-c.c. volumetric flask, made to volume with cold water, shaken thoroughly at intervals for about 30 minutes, allowed to settle for a short time, and the supernatant liquid tested for activity. The flask containing the suspension was kept continually in an ice bath.

*Preparation of substrate.*—The substrate used in all determinations was a soluble starch prepared by the General Chemical Company. A quantity of the starch was obtained, mixed, and the moisture content determined. Sufficient starch, on the oven-dry basis, for a 2% suspension was mixed with cold water to form a paste, poured into boiling water, and boiled for 2 minutes. The starch dispersion was cooled, transferred to a volumetric flask, buffered at pH 4.7 with 0.2M  $\text{Na}_2\text{HPO}_4$  and 0.2M  $\text{NaH}_2\text{PO}_4$ , and made to volume at 37°–40° C. with water.

*Determination of reducing equivalent expressed as maltose.*—The original Hagedorn and Jensen (1923) micro method for determining blood sugar as modified to the macro scale by Blish and Sandstedt (1933) and adapted to the determination of diastatic activity by Gore and Steele (1935) has been used throughout these investigations. Because the protein content of starch is very low, the protein precipitation was eliminated. Martin (1938) has found that by increasing the  $\text{Na}_2\text{CO}_3$  content of the potassium ferricyanide reducing solution to 50 g. per liter, greater precision in the determination of maltose is obtained. This modification was incorporated into the following procedure.

In determining the saccharogenic power of soybeans, 50 c.c. of the substrate was measured into a 125-c.c. Erlenmeyer flask and placed in a thermostat maintained at 40° C. Sufficient time was allowed for the material to reach the temperature of the water bath. One c.c. of the enzyme suspension, as measured from an accurately calibrated one-c.c.

pipette, was added to the substrate, mixed thoroughly, and allowed to digest for exactly 30 minutes. To accurately control the time of digestion it was found advantageous to start the digestions at one-minute intervals. Just before removal of a 5-c.c. sample the pipette was filled with the digestion mixture and was rapidly discharged back into the digestion flask by air pressure. This was repeated a second time. By so doing, no appreciable cooling of the digestion mixture would result when the final sample was taken. About 20 seconds before completion of digestion, the mixture was drawn into the pipette and the upper meniscus adjusted to the mark. Exactly at the end of 30 minutes the sample was delivered into 25 c.c. of the alkaline ferricyanide reducing solution. The 5-c.c. pipette used was so calibrated as to deliver 5 c.c. of water at 40° C. in 10 seconds. The samples were then heated in a boiling water bath for 15 minutes, cooled to 20° C., 25 c.c. of the acetic acid- $\text{ZnSO}_4$  reagent and 5 c.c. of 50% KI added, and titrated immediately with  $\text{Na}_2\text{S}_2\text{O}_3$ . Sufficient undigested starch remained in the digestion mixture to serve as the indicator.

The saccharogenic power of the soybean amylase concentrates was determined by a slight modification of the above procedure. With the amylase concentrates, 40 to 80 mg. of the dry material was suspended in the water by shaking in the volumetric flask rather than by grinding to a paste in an agate mortar. Five-tenths c.c. of the suspension so obtained was added to the substrate.

This method for determining the saccharogenic power has been found to be more precise and more easily adapted to general laboratory work than the usual methods for determining amylase activity.

*Variation of amylase content during germination.*—Because of a rapid development of molds, special precautions must be taken during germination. Removal of all broken or discolored beans, followed by fumigation with carbon disulphide according to the method described by Naylor and Dawson (1936) gave a bean which could be germinated satisfactorily in the light at 18°–20° C. without mold growth. The beans were spread in a single layer on moistened blotting paper in enameled pans and placed in a small glass hood to prevent excess dust contamination. Samples were removed at 24 hour intervals, crushed on a glass plate, dried for 12 hours in a stream of air maintained at 40–45° C., and stored in tightly stoppered bottles. Crushing the beans was essential to rapid and adequate drying. The dried samples were ground in a Wiley mill to pass a 40 mesh screen, and the saccharogenic power determined.

Three series of samples, each covering a six-day germination period, were tested. For comparative purposes the saccharogenic power (milligrams of maltose produced per milligram of air-dry bean) has been

calculated. These results are presented in Figure 1. It is evident from the curve of the average values that the available amylase in soybeans tends toward a slight decrease as germination proceeds. The marked increase of available amylase in other seeds during germination is shown by the data of Naylor and Dawson (1936). In their investigations the saccharogenic power varied from 5.1 in ungerminated oats to a maximum of 23.9 on the seventh day of germination. This compares with an average of 27.0 for ungerminated soybeans and an average of 25.2 on the sixth day of germination. Germination of soybeans was not carried further than six days because of the development of molds and the rapid decomposition of the beans beyond that period.

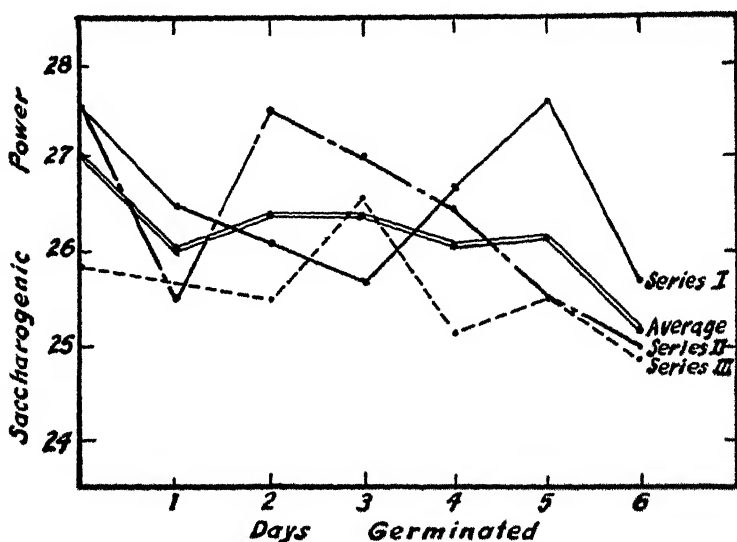


Fig. 1. Variation in saccharogenic power of soybeans during germination.

*Preparation of amylase concentrates.*—Water extraction of ground soybeans (40 mesh) followed by fractional precipitation with ethyl alcohol has shown that the greater portion of the enzyme is precipitated at an alcohol concentration of 65% by volume. Addition of alcohol to 85% yielded a concentrate only very slightly active.

Removal of these precipitates by filtration is a tedious and nearly impossible task. When they are thus obtained the precipitates are highly contaminated with filter paper. Only by adopting a technique based on centrifugation in a Sharples supercentrifuge has it been possible to obtain uncontaminated products in satisfactory yields. Further trouble was also caused by the oil-water emulsions formed when the soybeans were shaken with water during the extraction. These emul-

sions were entirely eliminated by first extracting the beans with ether in a modified Soxhlet extractor. Investigations of the amylase content of the soybeans before and after extraction have shown that the amylase present in the meal is only very slightly diminished during extraction with ether. Tests on the ether-free oil have failed to show any traces of amylase activity. After further studies of various phases of the preparation of the enzyme concentrate, the following procedure was adopted.

Good-quality, cured soybeans were ground in a Wiley mill to pass a 40-mesh screen. Two kilo lots of the ground beans were extracted about 20 times with diethyl ether in a modified Soxhlet extractor. The excess ether was allowed to evaporate at room temperature from the residue after extraction. Five hundred grams of the ether-extracted beans were covered with two liters of cold 50% alcohol. The temperature was maintained at that of running tap water (about 15° C.). The material was shaken at intervals for about three hours and then filtered through a double layer of cheese cloth, removing as much liquid as possible from the solid residue. This rough filtration was followed by centrifugation in a Sharples supercentrifuge at 20,000 r.p.m. The clear centrifugate was then cooled to 0° C. in an ice bath, and sufficient ice-cold absolute alcohol was added slowly, with continuous stirring, to raise the alcohol concentration to 65% by volume. The preparation was allowed to stand with occasional stirring for 15 minutes. The precipitate was then removed by centrifugation at 20,000 r.p.m., the precipitate collecting in the lower part of the bowl as a gelatinous, straw-colored film. This material was collected on a watch glass, dried under vacuum over  $\text{CaCl}_2$ , ground in an agate mortar, placed in a stoppered vial, and stored at a temperature of about 5° C. Based on the dry weight of the ether-extracted bean, yields of from 0.3 to 0.5% of enzyme concentrate were obtained. The saccharogenic power (milligrams maltose produced per milligram concentrate used) varied from 300 to 1360.

If in the foregoing procedure the alcohol concentration for precipitation is raised to 70% by volume, a much larger quantity of concentrate is obtained, but the saccharogenic power is almost quantitatively reduced. However, the concentrate so obtained is more soluble in water than the concentrate precipitated by 65% alcohol. For work other than concentration studies the more soluble preparation is of greater general use.

Although satisfactory yields of the amylase concentrate are obtained by the above method, only from 10 to 18% of the total amylase present in the beans is isolated as a solid concentrate. The task of improving this yield lies in future work.

*Characterization of soybean amylase.*—The enzyme concentrate prepared from soybeans was known to hydrolyze starch to products of high reducing value. To definitely establish the enzyme as an amylase, it was deemed necessary to identify maltose as a final degradation product. Fractionation of a typical digestion resulted in the isolation of a crystalline sugar and a dextrin-like material. This sugar was identified as maltose by its optical rotation and by the preparation and properties of two derivatives, the osazone and the octaacetate.

Following Kuhn's (1925) procedure, mutarotation studies of products formed in the early stages of hydrolysis of a substrate by soybean amylase show a positive mutarotation. The positive mutarotation shows an excess of  $\beta$ -maltose in the early stages of hydrolysis, and the enzyme is therefore classed as  $\beta$ -amylase. Giri's (1934) method of characterization also shows soybean amylase to be chiefly  $\beta$ -amylase.

Repeated determinations show that the 65% alcohol precipitates from soybean have a relatively high saccharogenic power. As compared with enzyme material prepared from rye and wheat by Naylor, Spencer, and House (1925) with a saccharogenic activity of 150 to 450, the enzyme concentrates prepared from soybean have saccharogenic powers of 300 to 1360. Application of Creighton and Naylor's (1933) modification of the Wohlgemuth method for the determination of amylolytic power to representative samples of all fractions obtained in the concentration of soybean amylase gave values so low as to show approximately no  $\alpha$ -amylase activity.

### Discussion

Early investigations of soybean amylase point toward its identity as  $\beta$ -amylase. Teller's more recent data indicate that the starch-splitting enzyme of soybeans contains both  $\alpha$ - and  $\beta$ -amylase. The work in this laboratory lends support to its characterization as  $\beta$ -amylase, with only a possible slight trace of  $\alpha$ -amylase, both before and after germination. The only evidence that points toward the presence of  $\alpha$ -amylase in soybean amylase is the marked ability of the concentrate to reduce the viscosity of starch paste. However, when we consider that the enzyme is one of the most powerful sugar-forming plant diastases, this sugar being formed by the hydrolysis of starch, it is not disturbing to observe a marked change in viscosity of the substrate during digestion with soybean amylase. More information as to the action of this enzyme on starch substrates is being furnished by work now in progress in this laboratory.

Such a convenient source of  $\beta$ -amylase concentrates in appreciable quantities as is furnished by the soybean is of considerable value in furnishing a tool for the characterization of starches and the degrada-

tion products derived therefrom. Martin (1938) and Martin and Newton (1938) have employed soybean amylase in such studies.

If concentration methods can be improved to insure better yields of the enzyme it is possible that soybeans may be used as a source of amylase preparations. Because of a decrease in amylase content during germination of the beans, germination will necessarily be eliminated from the preparative procedures. No special technical difficulties would be involved in preparing the concentrates, as concentration is a simple procedure when centrifugation is employed instead of filtration.

### Summary

Methods are given for determination of the saccharogenic power of soybeans and soybean-amylase concentrates. These methods are adaptable to the same determination in other seeds.

The amylase content of soybeans decreases slightly during germination.

A method for preparing amylase concentrates from soybeans is given.

Characterization by various methods indicates that the concentrates contain principally  $\beta$ -amylase.

### Acknowledgment

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## A STUDY OF GLUTEN PROTEIN FRACTIONATION FROM SODIUM SALICYLATE SOLUTION. PART III. THE EFFECT OF PROTEOLYTIC ENZYMES

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In a recent publication the present writer (1938b) presented the results of a study of the effects of proteoclastic action upon wheat glutens washed from doughs and dispersed in sodium salicylate solution. Marked differences in the quantity of gluten dispersed in the early period of exposure to sodium salicylate, following the incorporation of proteolytic enzymes in the dough mix, were demonstrated. At that time I presented a review of the more pertinent literature dealing with proteolytic action in flour doughs and suspensions, and in view of this fact, as well as in consideration of the comprehensive lists of citations which have been published from time to time, I will not review the literature to any extent in the present article. The application of gluten protein fractionation from sodium salicylate dispersions by successive additions of  $MgSO_4$  to the determination of gluten quality has been published (Harris, 1937, 1938a). This method has the advantage of rendering possible the recovery of the dispersed gluten in an apparently undenatured, unchanged condition, in contrast to the denaturing action of the classical solvents, dilute acid and alkali.

### Experimental Material and Methods

A hard red spring wheat flour commercially milled from 1936 crop wheat was used in this investigation. This flour was a bleached first patent containing 13.1% protein and 0.40% ash, and had already been employed in determining the effect of treatments of proteolytic enzymes upon the solubility in sodium salicylate of the gluten washed from dough containing the customary dough ingredients plus enzyme and prepared in the usual manner. The procedure followed in the present instance was essentially the method outlined in the study of protein solubility, and consisted in washing the gluten from doughs prepared from 50 grams of flour plus dough ingredients, under a small standardized stream of 0.1% sodium phosphate solution (Dill and Alsberg, 1924). The concentration of gluten dispersed in 10% sodium salicylate was gradually stepped up until a concentration of 6 g. per 100 cc. was reached. This concentration was afterward used in this work. The procedure now followed was to place 12 grams of a finely divided gluten in 200 cc. of 10% sodium salicylate solution contained in a 250 or 300 cc. Erlenmeyer flask, and disperse with frequent shaking. Several days were allowed for the dispersion process to take place.

When dispersal appeared to have reached an end the dispersions were centrifuged, aliquoted, and 25 cc. portions were placed in 50 cc. centrifuge tubes and the protein progressively fractionated by the successive additions of 1.5, 4, and 10 cc. of concentrated  $\text{MgSO}_4$  solution. Each fraction was removed by centrifuging from the residual liquid, washed with sodium salicylate plus  $\text{MgSO}_4$  of the same concentration as the liquid from which the fraction had been precipitated, and transferred to Kjeldahl flasks for the determination of the quantity of protein contained in the fraction. All determinations of these fractions were run in triplicate. The quantity of wet crude gluten washed from each dough was also ascertained, as well as the moisture content of the gluten and the percentage of dry gluten. Drying was done in an electric oven at  $105^\circ\text{C}$ . for 48 hours. A large quantity of  $\text{MgSO}_4$  solution was used in this study as compared with the quantity used in former work in order to remove as large a portion of the protein left in solution as possible. Even this increased addition did not completely clear up the solution when substantial quantities of certain proteoclastic enzymes had been added to the dough mix.

### Discussion

The data obtained from a preliminary investigation of the effects of proteolytic enzymes upon gluten fractionation are shown in Table I. Papain was the enzyme incorporated in the dough mix in this instance.

A concentration of 4 grams of gluten to 200 cc. of 10% sodium salicylate solution was used. The addition of papain apparently increased the moisture content of the washed gluten, but had no definite effect upon the percentage of dry gluten itself. No marked trend was evident in respect to total quantity of gluten soluble in the dispersions and enzyme concentration in the dough. A decrease of gluten removed as fraction I is shown with increasing papain dosages. Fraction II tends to increase slightly, while fraction III shows a decided increase with greater proteolytic activity in the dough.

TABLE I

THE EFFECT OF ADDITIONS OF PAPAIN UPON THE RELATIVE DISTRIBUTION OF GLUTEN PROTEINS FRACTIONATED FROM SODIUM SALICYLATE SOLUTIONS

Treatment	Mois- ture	Wet crude gluten	Dry gluten	Solu- bility	Frac- tion I	Frac- tion II	Frac- tion III	Total protein fractions	Percent of total soluble
	%	%	%	mg.	mg.	mg.	mg.	mg.	
Control	68.8	42.9	13.4	461	137	209	40	386	83.7
Papain, 0.003%	69.2	42.2	13.9	499	115	232	54	401	83.7
Papain, 0.006%	69.8	42.9	13.0	493	102	244	56	402	81.5
Papain, 0.010%	70.6	44.8	13.2	461	74	236	70	380	82.4

Concentration of gluten 4 grams per 200 cc. of dispersion.

Solubility and protein fractions are computed for Tables I, II, and III on the basis of 100 cc. of dispersion. Fractions are the quantity of protein removed by 6, 16, and 40 cc. of concentrated  $\text{MgSO}_4$  solution, respectively.

In Table II the data obtained with papain as the added proteolytic agent revealed a very similar situation when a gluten concentration of 8 grams to 200 cc. of dispersion was used. A marked increase in fraction II is shown with the papain treatments. The decrease in fraction I is quite evident, as well as a corresponding increase in fraction III. A higher percentage of total protein appears to be removed from dispersion when the concentration of the dissolved gluten is increased.

TABLE II

THE EFFECT OF ADDITIONS OF PAPAIN UPON THE RELATIVE DISTRIBUTION OF GLUTEN PROTEINS FRACTIONATED FROM SODIUM SALICYLATE SOLUTIONS

Treatment	Mois- ture	Wet crude gluten	Dry gluten	Solu- bility	Frac- tion I	Frac- tion II	Frac- tion III	Total protein fractions	Percent of total soluble
	%	%	%	mg.	mg.	mg.	mg.	mg.	
Control	67.4	38.0	12.4	855	286	413	58	757	88.5
Papain, 0.006%	69.8	40.4	12.3	992	264	535	74	873	94.7
Papain, 0.012%	70.4	47.3	14.0	940	187	527	79	793	84.4

Concentration of gluten 8 grams per 200 cc. of dispersion.

In Table III more comprehensive data are shown relative to the effect of a number of proteolytic enzymes upon gluten protein fractionation. The concentration of gluten in the work reported in this table was raised to 12 grams per 200 cc. of dispersion. Examining the papain extraction data first, it is evident that the increase in the quantity of gluten dispersed did not materially alter the effect produced by the papain additions upon the relative gluten fractions. A general rise in moisture content of the wet crude gluten and a decrease in percentage of dry gluten is evident with the higher treatments of papain. There is also evidence of increased gluten solubility with higher enzyme concentrations, although this solubility appears to become less as the highest papain treatments were used. A marked trend toward reduction of the protein removed as fraction I is shown as the enzyme concentration is raised. The second fraction tends to increase, then de-

TABLE III

THE EFFECT OF PROTEOLYTIC ENZYMES UPON THE RELATIVE DISTRIBUTION OF GLUTEN PROTEINS FRACTIONATED FROM SODIUM SALICYLATE SOLUTIONS

Sample	Moisture	Wet crude gluten	Dry gluten	Solubility	Fraction I	Fraction II	Fraction III	Total protein fractions	Percent of total soluble
	%	%	%	mgs.	mgs.	mgs.	mgs.	mgs.	
Control	67.1	39.6	13.3	1245	415	610	69	1094	87.9
Papain, 0.002%	68.1	43.7	13.9	1318	383	704	69	1156	87.7
Papain, 0.004%	68.8	44.6	13.9	1342	293	709	133	1135	84.6
Papain, 0.006%	66.3	37.0	12.5	1533	255	924	105	1284	83.8
Papain, 0.010%	69.4	39.2	12.0	1536	319	857	150	1320	85.9
Papain, 0.020%	69.2	39.0	12.0	1447	173	739	435	1347	93.1
Papain, 0.040%	71.4	41.5	11.9	1456	84	483	240	807	55.4
Papain, 0.060%	—	—	—	1323	73	210	492	775	58.6
Yeast water, 10 cc.	70.7	45.1	13.2	1325	524	593	53	1170	88.3
Yeast water, 20 cc.	71.9	42.7	12.0	1368	535	628	60	1223	89.4
Yeast water, 30 cc.	71.1	41.8	12.1	1378	521	636	53	1210	87.8
Pepsin, 0.04%	69.0	43.4	13.5	1143	395	524	103	1022	89.4
Pepsin, 0.12%	69.9	43.6	13.1	1354	494	634	83	1211	89.4
Pepsin, 0.20%	70.6	45.4	13.4	1316	448	638	77	1163	88.4
Pepsin, 0.30%	71.9	45.9	13.0	1277	415	650	74	1139	89.2
Pepsin, 0.50%	71.2	47.8	13.8	1348	415	680	114	1209	89.7
Pepsin, 1.00%	71.0	39.3	11.4	1516	452	805	75	1332	87.9
Pancreatin, 0.01%	67.3	40.1	13.1	1436	470	775	64	1309	91.2
Pancreatin, 0.02%	65.5	39.2	13.5	1516	452	805	75	1332	87.9
Pancreatin, 0.03%	68.1	41.2	13.1	1414	394	775	103	1272	89.9
Pancreatin, 0.04%	67.2	38.4	12.6	1493	406	830	96	1332	89.2
Malt diastase, 0.5%	68.6	42.9	13.2	1379	274	768	139	1181	85.6
Malt diastase, 1.0%	69.7	44.2	13.4	1385	194	734	224	1152	82.9
Taka diastase, 0.5%	68.7	40.2	12.6	1442	150	636	107	893	61.9
Taka diastase, 1.0%	69.6	41.4	12.6	1553	52	472	294	818	52.7

Concentration of gluten 12 grams per 200 cc. of dispersion.

crease, while fraction III increases sharply with added enzymes. The total protein removed from dispersion increases at first and then falls sharply later.

A different picture is presented by a study of the yeast-water data. Here we are presumably dealing with a protease activator, and the enzyme involved is a natural flour protease, which has been considered by many workers to be akin in nature to papain. The effects of these proteases upon wet crude gluten moisture and dry gluten percentages are similar to the effects of papain, the moisture content increasing and dry gluten percentage decreasing as more activating agent is added, while the solubility of the washed gluten is also increased as compared with the control. A very marked difference, however, is evident when the values obtained for fraction I by the two enzyme actions are compared. No evidence of a fall in the quantity of protein precipitated is shown with yeast-water increase, but actually an increase when compared with the quantity removed in the control. Fractions II and III, as well as the total protein removed from the dispersion, show no great differences for the different treatments.

In the instance of pepsin the same trend is visible in moisture content, dry gluten percentage, and, to some extent, in gluten solubility. No definite trends appear to be established in the case of fractions I and III, but fraction II clearly increases with addition of enzyme. Thus pepsin differs in its effect upon the distribution of these gluten protein fractions from both papain and flour protease, as would be expected from its source as an animal enzyme, whereas the two previous enzymes are of plant origin.

Pancreatin shows the same effect upon moisture, dry gluten, and gluten solubility as the other enzymes already considered, but resembles pepsin in its action upon fraction I. Pancreatin is more effective on a percentage basis than pepsin, although it belongs to the same class of enzyme. This enzyme also increases the protein removed in fraction II as compared with the control.

When the diastases are considered, the same relationships between enzyme concentrations and moisture, dry gluten, and gluten solubility are shown. A decided decrease in fraction I was yielded by both enzymes, but the effect of the two enzymes varies when fraction II is considered. Malt diastase tends to increase fraction II, but taka diastase apparently reduces the quantity of protein removed at this point of the fractionation process when present in substantial quantities. There are also significantly lower quantities of protein removed from dispersion when the doughs treated with taka diastase are considered, as compared with the other proteolytic agents, with the exception of the two highest treatments of papain.

When Table IV, which contains the gluten and fractionation data arranged as percentages of the control dough, is examined, these conclusions are brought out even more clearly. The moisture content of wet gluten is raised in some instances to 107% of the control by enzyme treatments, while a decrease in dry gluten is evident in the majority of the cases. Increases in gluten solubility range as high as 125% of control and in further investigations of this nature it would seem advisable to insure a constant level of dispersed gluten concentration. Changes in the percentages shown in fraction I are especially striking, running in one instance as low as only 12% of the control without enzyme treatment. Glutathione-treated doughs show marked increases in this fraction, and differ from the other data in this respect. Papain and pancreatin appear to have the greatest effect in increasing the quantity of protein thrown down as fraction II, while yeast water and taka diastase have the least effect. Papain also greatly increases

TABLE IV

THE EFFECT OF PROTEOLYTIC ENZYMES UPON MOISTURE CONTENT AND WEIGHT OF THE WASHED GLUTEN, AND THE DISTRIBUTION OF THE GLUTEN PROTEINS

Data Calculated as Percentages of the Untreated Control

Sample	Moisture	Wet crude gluten	Dry gluten	Solubility	Fraction I	Fraction II	Fraction III	Total fractions
	%	%	%	%	%	%	%	%
Papain, 0.002%	101	110	104	106	92	115	100	106
Papain, 0.004%	102	113	104	108	71	116	193	104
Papain, 0.006%	99	93	94	123	61	151	152	117
Papain, 0.010%	103	99	90	123	77	140	217	121
Papain, 0.020%	103	98	90	116	42	121	630	123
Papain, 0.040%	106	105	89	117	20	79	348	74
Papain, 0.060%	—	—	—	106	18	34	713	71
Yeast water, 10 cc.	105	114	99	106	126	97	77	107
Yeast water, 20 cc.	107	108	90	110	129	103	87	112
Yeast water, 30 cc.	106	106	91	111	125	104	77	111
Pepsin, 0.04%	103	110	102	92	95	86	149	93
Pepsin, 0.12%	104	110	98	109	119	104	120	111
Pepsin, 0.20%	105	115	101	106	108	105	112	106
Pepsin, 0.30%	107	106	101	103	100	107	107	104
Pepsin, 0.50%	106	121	98	109	100	111	165	110
Pepsin, 1.00%	106	99	85	122	109	132	109	122
Pancreatin, 0.01%	100	99	98	115	113	127	93	120
Pancreatin, 0.02%	98	99	102	122	109	132	109	122
Pancreatin, 0.03%	101	104	98	114	95	127	149	116
Pancreatin, 0.04%	100	97	95	120	98	136	139	122
Malt diastase 0.5%	102	92	99	90	66	126	201	108
Malt diastase 1.0%	104	112	101	111	47	120	325	105
Taka diastase 0.5%	102	101	95	116	36	104	155	82
Taka diastase 1.0%	104	104	95	125	12	77	426	75

TABLE V

THE EFFECT OF PROTEOLYTIC ENZYMES UPON GLUTEN PROTEIN FRACTIONATION  
FRACTIONS CALCULATED AS PERCENTAGES OF SOLUBLE PROTEIN

Sample	Fractions			Total protein removed
	I	II	III	%
Control	33.3	49.0	5.5	87.9
Papain, 0.002%	29.1	53.4	5.2	87.7
Papain, 0.004%	21.8	52.8	9.9	84.6
Papain, 0.006%	16.6	60.3	6.8	83.8
Papain, 0.010%	20.7	55.8	9.8	86.3
Papain, 0.020%	12.0	51.0	30.1	93.1
Papain, 0.040%	5.8	33.2	16.5	55.4
Papain, 0.060%	5.5	15.9	37.2	58.6
Yeast water, 10 cc	39.5	44.8	4.0	88.3
Yeast water, 20 cc.	39.1	45.9	4.4	89.4
Yeast water, 30 cc.	37.8	46.2	3.8	87.8
Pepsin, 0.04%	34.6	45.8	9.0	89.4
Pepsin, 0.12%	36.5	46.8	6.1	89.4
Pepsin, 0.20%	34.0	48.5	5.9	88.4
Pepsin, 0.30%	32.5	50.9	5.8	89.2
Pepsin, 0.50%	30.8	50.4	8.5	89.7
Pepsin, 1.00%	29.8	53.2	4.9	87.9
Pancreatin, 0.01%	32.7	54.0	4.5	91.2
Pancreatin, 0.02%	29.8	53.1	4.9	87.8
Pancreatin, 0.03%	27.9	54.8	7.3	90.0
Pancreatin, 0.04%	27.2	55.6	6.4	89.2
Malt diastase 0.5%	19.9	55.7	10.1	85.7
Malt diastase 1.0%	14.0	53.0	16.2	83.2
Taka diastase 0.5%	10.4	44.1	7.4	61.9
Taka diastase 1.0%	3.3	30.4	18.9	52.7

the protein removed as fraction III, differing very markedly from the other enzymes examined in this respect. Yeast water, on the other hand, depresses the quantity of protein removed at this point. It is possible that increased severity of treatment with some of the other enzymes investigated, especially the diastases, might have the same effect in raising the final fraction and would therefore correspond to the effect of papain. Papain was used throughout in small dosages as compared with the relatively large concentrations of diastase employed in this study.

The fractionation data were recalculated and expressed as percentages of protein found to be soluble in the various dispersions. These calculations were made to obviate the possible influence of high gluten concentrations on the fractionation results. These values are shown in Table V. The data in this table yield much the same picture as

was presented in the former table, and emphasize the decrease in fraction I with substantial treatment of papain, malt diastase, and taka diastase. Papain and taka diastase both affected the quantity of protein removed from solution.

The effect of various proteolytic agents incorporated in dough upon the properties of gluten washed from these doughs may be summarized as follows: Proteolytic enzymes in general tend to increase the moisture content of the washed crude gluten, to decrease the percentage of dry gluten recovered from a dough, and to increase the solubility of the gluten in 10% sodium salicylate solution. These enzymes differ greatly among themselves, however, in their respective effects upon the relative distribution of the protein fractions of the dispersed gluten. Papain, malt, and taka diastase have been shown to have a very striking effect in reducing the quantity of gluten protein contained in fraction I. The flour proteases, when activated by glutathione, tend to increase the quantity of protein removed in fraction I, while pepsin and pancreatin do not have much effect. Initial treatments of papain raise the protein content of fraction II, but heavier additions cause a decrease. Malt diastase increases this fraction, while taka diastase appears to assert a depressive action, especially when present in 1.0% concentration. Flour protease is without appreciable effect upon fraction II, pepsin tends to raise this fraction with heavier dosages, and pancreatin increases fraction II at all concentrations used.

Fraction III is greatly augmented by increasing concentrations of papain, and the same effect is to be observed for both diastases studied in this investigation. Flour proteases, when activated by glutathione, are the only enzymes which appear to show a reducing effect upon this fraction. The total protein removed from these dispersions is increased by proteolytic action, with the exception of the heavier dosages of papain, and by taka diastase. Flour protease appears to differ distinctly from papain in its effect upon gluten fractionation, and the results obtained in this study, as well as from work reported by the author regarding the inhibiting effect of  $\text{KBrO}_3$ , would not justify their being classified in the same group of proteolytic enzymes. This is in opposition to certain statements which have appeared in the literature but agrees with the findings of Read and Haas (1937a), who postulated no repressive action of  $\text{KBrO}_3$  upon flour proteases, as opposed to marked inhibition of papain and bromelin.

It is probable that papain, malt diastase, and taka diastase, in the concentrations employed in this study, exert a dispersive effect upon the gluten protein complex, causing the formation of smaller particles of gluten protein in sodium salicylate dispersion. Consequently the



quantity of protein removed from fraction I, which supposedly consists of the larger particles or micelles present in the dispersion, is decreased. As the protein removed in fraction I falls, more protein is removed in fraction II, as a result of an increase in the number of particles fractionated here because of a breaking down of the larger-size or first-fraction micelles. As the proteolytic action is stepped up, however, further disruptions of the gluten complex occur, and fraction II is diminished in quantity. This effect may be carried through fraction III, causing a smaller proportion of protein to be precipitated here, and may even affect the total quantity of protein removed because of inability to precipitate out these smaller particles from the dispersion by addition of  $\text{MgSO}_4$  and centrifuging. The use of yeast water appears to induce a coagulative effect upon the flour gluten, causing more protein to be precipitated in fraction I, with less protein removed in the third fraction. Pepsin did not greatly alter the relative distribution of the fractions; at least no definite trends are apparent. These facts correspond with the theory that pepsin acts chiefly upon peptone and peptides. Pancreatin appeared to change the distribution of protein slightly, having first a coagulative, then a slight dispersive effect.

It is extremely probable that a shift in the pH of a dough would cause appreciable changes in the effect of the enzymes included in this study. A complicating factor in setting up an investigation of the action of proteolytic enzymes upon dough protein at various pH levels is the effect of these changes upon the characteristics of the flour gluten itself. It is probably also true that a change in dough temperature would cause changes in the relative effects of these various enzymes upon the properties of the gluten protein.

The conclusions reached in this study are essentially in agreement with results reported by Read and Haas (1934, 1936), Bohn and Bailey (1937), and Rupp and Bailey (1937) when investigating the effect of proteolytic enzymes upon dough and protein characteristics. These workers agree upon the protein-attacking power of proteolytic enzymes as registered in their liquefying effect upon gelatin, as well as in decreasing dough resistance to mechanical stress. No definite evidence was adduced in the present research to indicate a coagulative effect upon the gluten proteins by small additions of papain, as suggested by Balls and Hale (1936), but it is possible that this effect was produced by other proteolytic enzymes studied.

It is probable that proteolytic enzymes in general exert a progressive degrading action upon the gluten complex in breaking it down to smaller portions. This effect is reflected in the relative quantities of

protein successively fractionated, with a shift toward the region of smaller particles. This general result of proteolytic action might suggest that gluten protein is composed of one individual complex which may be split into almost innumerable fractions, these fractions being greatly affected in their distribution by proteolytic action. Other evidence obtained previously by the author and already published, however, appeared to indicate the presence of three distinct gluten complexes which differ among themselves in physical and chemical properties. The result of enzymic action on such a system would be that a mixture of these principal components would be obtained in the three fractions with each individual mixture varying with the enzyme treatment used. Physical differences in corresponding fractions caused by these treatments were very noticeable in many instances, and lend support to the theory, inasmuch as these separations did not always appear to consist of one homogeneous substance.

### Summary and Conclusions

A series of 50-gram doughs were mixed from a hard red spring wheat flour, including simple basic-formula ingredients plus various proteolytic enzymes. These doughs were mixed in the usual manner, and the gluten immediately washed therefrom. These glutes were then dispersed as completely as possible in 10% sodium salicylate, and the protein fractionated by successive additions of appropriate quantities of  $MgSO_4$ . The protein content of these fractions was determined, and the quantity contained in the different fractions computed. A consideration of the summarized data would appear to justify the following conclusions:

Proteolytic enzymes added to dough may profoundly affect the relative distribution of the gluten protein fractionated from the washed-gluten dispersion of such doughs.

Various types of proteolytic enzymes affect the protein distribution in different ways, and marked dissimilarities are shown between different enzymes.

Papain, malt diastase, and taka diastase show evidences of a disruptive action upon the gluten complex, causing the formation of smaller protein particles in the dispersion, with consequent shifts in the quantities of protein contained in the various fractions.

Flour proteases, when activated by glutathione in concentrations present in the yeast water used in this work, appear to coagulate rather than disperse the gluten complex. Pancreatin may have a similar effect when present in moderate amounts. No definite trends were established from a study of the data obtained by the use of pepsin.

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## IMMEDIATE EFFECT OF CROSS POLLINATION ON THE CAROTENOID PIGMENTS IN THE ENDOSPERM OF MAIZE <sup>1</sup>

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From previous investigation on carotenoid pigments in the endosperm of maize, it was shown that among inbred lines striking variations in concentrations of total carotenoid pigments occur.<sup>3</sup> To investigate further the magnitude of these differences, a survey was made in 1937 with a larger series of inbred lines. A study was made also to determine the immediate effect on the endosperm of cross

<sup>1</sup> Contribution from the Division of Agronomy and Plant Genetics and the Department of Botany, University of Minnesota. Paper No. 1633 of the Journal Series. Minnesota Agricultural Experiment Station.

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<sup>3</sup> I. J. Johnson and Elmer S. Miller, Variation in carotenoid pigment concentration among inbred and crossbred strains of corn, *Cereal Chem.* 15: 345-350, 1938.

pollination between groups of high and low carotenoid-pigment strains. From the analysis of carotenoid pigments in the leaf tissue of the lines used in this study, it was possible to determine the relationship between the concentration of these pigments in the endosperm to that in the leaf tissue.

### Material and Methods

The analysis of carotenoid-pigment concentration in the endosperm and in the leaf tissue was made with a group of 35 inbred lines of dent corn used in the corn-improvement program. These inbred lines had been inbred for several generations and were relatively homozygous for visible morphological characters. The seed of the inbred lines used for analysis was produced by sib pollination. A large number of crosses had been made in 1937 among these lines which provided the opportunity to study the immediate effect of cross pollination with lines that differed widely in concentration of endosperm carotenoids.

The seed, containing from 7% to 9% moisture, was finely ground in a Wiley mill until the meal passed through a 300-mesh sieve. No significant heating effects were observed when thus ground. The meal was tempered for 30 minutes as aqueous paste, and the final extraction was made by repeated grinding with sand in a mortar. After two extractions with acetone, followed by three to five with diethyl ether, the extractions were combined and transferred to a separatory funnel containing 150 c.c. of water. After the acetone in the extract was removed by two additional extractions with water, the solutions were made up to volume in ether.

The determination of total carotenoid-pigment concentration was made spectrophotometrically as described by Miller.<sup>4</sup>

### Experimental Results

*Variations in carotenoid pigment between strains.*—Among the 35 inbred lines studied, a range from 0.89 to 2.57 mg. % total carotenoid pigments was obtained. Duplicate samples were analyzed from 15 strains to determine the extent of variability and to obtain a measure of the magnitude of the standard error. From the analysis of variance given in Table I, the F value of 116.0 greatly exceeds the one-percent point, indicating that the strains differed significantly in percentage of total carotenoid pigments. The low standard error of the mean of 0.0419 mg. % indicates that the method employed in analysis is very accurate. On the basis of the commonly accepted odds of 19 : 1 for significance, strains differing by 0.118 mg. % total carotenoid represent significant differences.

<sup>4</sup> Elmer S. Miller, Photoelectric spectrophotometry applied to the quantitative analysis of carotenoid and chlorophyll pigments in ternary and quaternary systems, *Cereal Chem.* 15: 310-316, 1938.

TABLE I  
ANALYSIS OF VARIANCE OF 15 DUPLICATE SAMPLES FOR PER CENT CAROTENOID  
PIGMENTS IN THE ENDOSPERM OF MAIZE

Variance due to	D.F.	Sum sq.	Mean sq.	S.D.	F.
Varieties	14	5.6960	0.4069	—	116.0 <sup>1</sup>
Samples	1	0.0001	0.0001	—	—
Error	14	0.0491	0.003507	0.0592	—
Total	29	5.7452	—	—	—

<sup>1</sup> Exceeds the 1% point in level of significance. S. E. mean = 0.0419.

In previous studies with a smaller number of strains, an attempt was made to determine the relationship between visible endosperm color and the percentage of carotenoid pigments. The 35 lines analyzed in this study were classified into three groups: dark, medium, and light yellow endosperm color. The average percentage of total carotenoid pigments for each of the three groups is given in Table II.

TABLE II  
AVERAGE MG. PER CENT AND RANGE IN TOTAL CAROTENOID PIGMENTS IN THE  
ENDOSPERM OF 35 INBRED STRAINS CLASSIFIED AS DARK, MEDIUM, AND  
LIGHT YELLOW IN COLOR

Endosperm color	No. of strains	Range in mg. % total carotenoids	Average mg. % total carotenoids
Dark yellow	8	1.40-2.57	1.89±0.1317
Medium yellow	18	0.98-2.09	1.67±0.0651
Light yellow	9	0.89-1.91	1.47±0.1031

The difference between the means of the dark and medium yellow lines is 1.5 times the standard error, between the medium and light yellow lines 1.6 times the standard error, and between the dark and light yellow endosperm lines 2.5 times the standard error of a difference.

The differences in total carotenoids between the color classes are not large, although the difference between the mean of the dark-yellow and light-yellow group exceeds the 5% point in level of significance and would indicate that dark-yellow endosperm lines of corn are higher in carotenoid pigments than the light-yellow lines. The range in mg. % total carotenoids was relatively large within each of the three color classes, probably as a result of difficulties in making accurate endosperm color classifications because of other pigments such as "brown aleurone," often associated with yellow endosperm corn.

The results of this study would indicate the possibility of selecting inbred lines high in carotenoid pigments as a means of improving the feeding value of corn in respect to vitamin A.

*Immediate effect of cross pollination on carotenoid pigments.*—Among the 35 inbred lines analyzed, 10 were classified as high and 5

as low in carotenoid-pigment concentration. In respect to pigment content, crossed seed was available in the following combinations: high female  $\times$  high male, high female  $\times$  low male, low female  $\times$  high male, and low female  $\times$  low male.

These types of crosses provided an opportunity to study the immediate effect of cross pollination when high carotenoid lines were pollinated with high carotenoid male parents in contrast with pollinations with low carotenoid male parents. Similar effects of pollination could also be studied when low carotenoid female parents were pollinated by high and low male parents, respectively.

From the data of this study given in Table III, it is evident that the average percentage of total carotenoids in the endosperm of crossed seed is significantly influenced by the genes for carotenoid pigments contributed by the male gametes when the parents differ in total carotenoid pigment percentage.

TABLE III  
IMMEDIATE EFFECT OF CROSS POLLINATION ON THE PERCENTAGE OF  
TOTAL CAROTENOID PIGMENTS IN THE ENDOSPERM

Parents crossed -	No. crosses	Average mg. % total carotenoids			Increase or decrease over female parent	Odds for significance <sup>1</sup>
		Female parent sibbed	Male parent sibbed	Cross		
High female $\times$ high male	16	2.16	1.98	2.08	-0.08	9 : 1
High female $\times$ low male	11	2.13	1.14	1.79	-0.34	10,000 : 1
Low female $\times$ high male	7	1.30	1.98	1.58	0.28	600 : 1
Low female $\times$ low male	5	1.27	1.18	1.35	0.08	3 : 1

<sup>1</sup> Student's pairing method and tables of Z.

When high-carotenoid-pigment female parents were pollinated by high-carotenoid-pigment male parents, or when low-carotenoid-pigment females were pollinated by low male parents, the crossed seed was not significantly different from the female parent. However, when high-carotenoid-pigment female parents were pollinated by low-carotenoid male parents, the crossed seed was significantly lower in pigment concentration than that of the sibbed seed of the female parent. Similarly, the crossed seed from low-carotenoid-pigment females pollinated by high-carotenoid-pigment males was significantly higher in pigments than the female parent. Among the eleven crosses between high-carotenoid female parents and low-carotenoid male parents, the crossed seed was lower than the female parent in all cases and in each of the seven crosses between low females and high males, the crossed seed had a higher percentage of total carotenoid pigments than the female parent. These results

would suggest that carotenoid pigments in the endosperm are subject to the xenia effects often found in endosperm characters.

On the basis that two-thirds of the endosperm genotype for carotenoid pigments is contributed by the female gametes and one-third by the male gametes, a very close agreement was found between the calculated value for the carotenoid pigments in the crossed seed and the values actually obtained. In the first group of crosses (high female  $\times$  high male), the calculated value was 2.10 mg. % total carotenoids and the actual value was 2.08 mg. %. Similarly, in the crosses between high female  $\times$  low male, low female  $\times$  high male, and low female  $\times$  low male, the calculated and actual values were 1.80 and 1.79, 1.52 and 1.58, and 1.24 and 1.35 mg. % respectively.

In a study with 35 inbred lines of the relationship between the percentage of total carotenoids in the leaf tissue and in the endosperm, a correlation coefficient of 0.1418 was obtained. Since this correlation does not approach the 5% point in level of significance, in this group of material the percentage of carotenoids in the leaf tissue apparently has no significant relation to that found in the endosperm. From previous investigations, it was shown that the leaf tissue of white endosperm lines contains as much carotenoid pigments as those from yellow endosperm strains. Apparently, the factors responsible for the formation of carotenoid pigments in the leaf tissue are not associated with those for the development of these pigments in the endosperm tissue.

### Summary

A method is described which permits the quantitative extraction of carotenoids from maize endosperm.

Of the 35 inbred lines studied, it was found that significant variations occur with reference to carotenoid-pigment concentration.

When inbred lines were classified into dark, medium, and light yellow in endosperm color, the difference in total carotenoid pigments between the groups dark and medium, and medium and light, were not significant; but only the dark and light groups were statistically different.

When intercrosses were made between high female  $\times$  low male, and low female  $\times$  high male, the crossed seed was significantly different from the female parent, indicating that carotenoid pigments are subjected to the usual xenia effects.

No significant correlation was observed between the concentration of carotenoid pigments in the endosperm of inbred lines and that in the leaf tissues—suggesting that the two are due to independent factors or physiological processes.

## THE LEAVENING ACTION OF AIR INCLUDED IN CAKE BATTER

J. A. DUNN and J. R. WHITE

Lever Brothers Co., Cambridge, Mass.

(Read at the Annual Meeting, May 1938)

In a communication presented before the 1937 annual convention, the present authors (1937) discussed the relationship between creaming volume, batter volume, and cake volume of lean pound cakes containing no chemical leavening agents. It was shown that whereas the correlation between creaming volume and finished cake volume is positive (subject to rather wide variations), the correlation between batter volume and finished-cake volume is practically a straight-line function. Because lightness of batter is directly associated with finished-cake volume, it follows that the amount of air incorporated into the batter in one of several ways should have a direct bearing on the volume of the finished cakes.

Using the method described previously,<sup>1</sup> we measured the specific volumes (the reciprocal of the specific gravity) of 6 ingredients used in the pound-cake test. Table I shows the proportions of these 6 ingredients used in the pound-cake batter and the specific volume or measure of lightness for these same ingredients.

TABLE I  
PROPORTIONS AND SPECIFIC VOLUMES OF INGREDIENTS USED IN POUND-CAKE BATTER

	Weight used	Specific volume
	<i>g.</i>	
Sugar	900	1.15
Shortening	450	1.09
Salt	27	1.23
Eggs	450	0.97
Milk	500	0.966
Flour	900	1.75

If each of the foregoing amounts of ingredients is multiplied by its specific volume, it is apparent that the total volume of one batch of pound-cake batter should be 4,053 c.c. Dividing this figure by the total weight of the batch (3,227 g.), one obtains a specific volume of approximately 1.25. Without any mixing, this would be the theoretical lightness of a pound-cake batter, *if none of the ingredients*

<sup>1</sup> J. A. Dunn and J. R. White, Factor control in cake baking, *Cereal Chemistry* 14: 783-801, 1937.



*were soluble in or miscible with water.* Actually, the salt and sugar dissolve almost completely in the milk, with consequent loss in volume. Moreover, the flour absorbs liquid with consequent volume change.

An investigation of the leavening action of the air included in pound-cake batters seemed likely to result in some interesting data from which it was hoped some fundamental conclusions might be drawn.

### Experimental

A double batch of pound-cake batter was mixed, and a batter volume of 1.37 was obtained. Half of the finished batter was placed in a vacuum desiccator and a vacuum carefully applied intermittently, with care taken to avoid losing any of the batter through the vacuum system. After a short period of time, the batter ceased to give up any air, and by this time it had taken on an appearance somewhat like that of custard cream. Normal amounts of this evacuated batter and of the control batter were scaled and baked according to our standard procedure.

At the same time, another batch of pound-cake batter was made according to a special procedure designed to avoid the incorporation of air. This procedure was essentially as follows: All of the soluble ingredients were dissolved in the milk and eggs. The flour was placed in the mixing machine, the liquid was poured in, and the batter mixed at low speed with a dough hook. At the end of the total mixing time of  $1\frac{1}{2}$  minutes, melted shortening was added and mixing continued until the shortening was distributed. The specific volume of this specially mixed batter was 0.87. The specific volume of the control was 1.37. The specific volume of the pound-cake batter which had been evacuated was 0.80. Figure 1 shows the cakes which resulted.

The cake on the right represents the "absolute zero" for this pound-cake formula. The volume of the batter placed in the pan was 908 c.c., and the volume of the resulting baked cake was 902 c.c. In other words, this cake exhibited absolutely no leavening action. The cake in the center, although ruined from a practical point of view, did expand somewhat during baking. The volume of the batter before baking was 987 c.c.; thus the increase during baking was 430 c.c. It is evident, therefore, that we were unable to mix a cake batter that was entirely devoid of included air. In all probability air was introduced by the flour and sugar, and to a lesser degree by the milk and eggs.

Some months later, a large batch of pound-cake batter was mixed and three-fourths of this batter was evacuated as previously described. That portion of the batter which had not been evacuated was used as a control and was baked according to the standard procedure. A second portion which had been evacuated was scaled and baked ac-

cording to standard procedure. A third portion was placed in the mixer and mixing was continued at low speed until a maximum batter volume had been reached. The fourth (evacuated) portion was placed in the mixer and mixed at medium speed until maximum specific volume was reached. The specific volume of the normal batter was 1.31. The specific volume of the evacuated batter was 0.80. After the evacuated batter was mixed at low speed for 40 minutes, a specific volume of 1.26 was obtained. Further mixing did not increase the lightness of batter. The portion of evacuated batter that was mixed at medium speed reached a maximum batter volume of 1.28 in 30 minutes, after which subsequent mixing did not increase the lightness. A cold-water bath was used during this remixing, in order to prevent overheating of the batter. The resulting cakes are shown in Figure 2.

Although it was impossible to cream into an evacuated batter the same amount of air which the batter previously contained, we were able to approach the previous lightness more closely than had been expected. The cakes produced by the remixed batters showed distinct evidences of overdevelopment of the gluten. The crust and crumb were toughened somewhat and the cakes tended toward a rather bold contour. The cakes resulting from the batter remixed at medium speed also showed excessive holes.

A sample of shortening was evacuated in the same manner in which the pound-cake batters had been handled until all of the air had been exhausted. A standard pound cake was made from this evacuated shortening, with a sample of the original shortening as a control. A batter volume and cake volume somewhat lower than the standard were obtained, after which we then endeavored to make up for the lack of air in the shortening by increasing the creaming time by 20%. This extra creaming raised the batter volume until it closely approximated that of the control. The loaf volume was likewise raised, but did not quite approach the volume of the control.

Figure 3 shows the resulting cakes and it is apparent that the air introduced during the manufacture of shortening tends to improve the quality of cakes and to reduce the time necessary to mix the cake. It will be noted that the decrease in volume caused by removing the air from the shortening is chiefly accounted for by side shrinkage rather than by a decrease in the height of these cakes. Extra creaming straightened the sides and reduced the shrinkage.

### Discussion

On the basis of the data obtained, it has been possible to calculate how much of the volume increase is produced by the thermal expansion of the air included in the batter. The batter temperature was 78° F.,

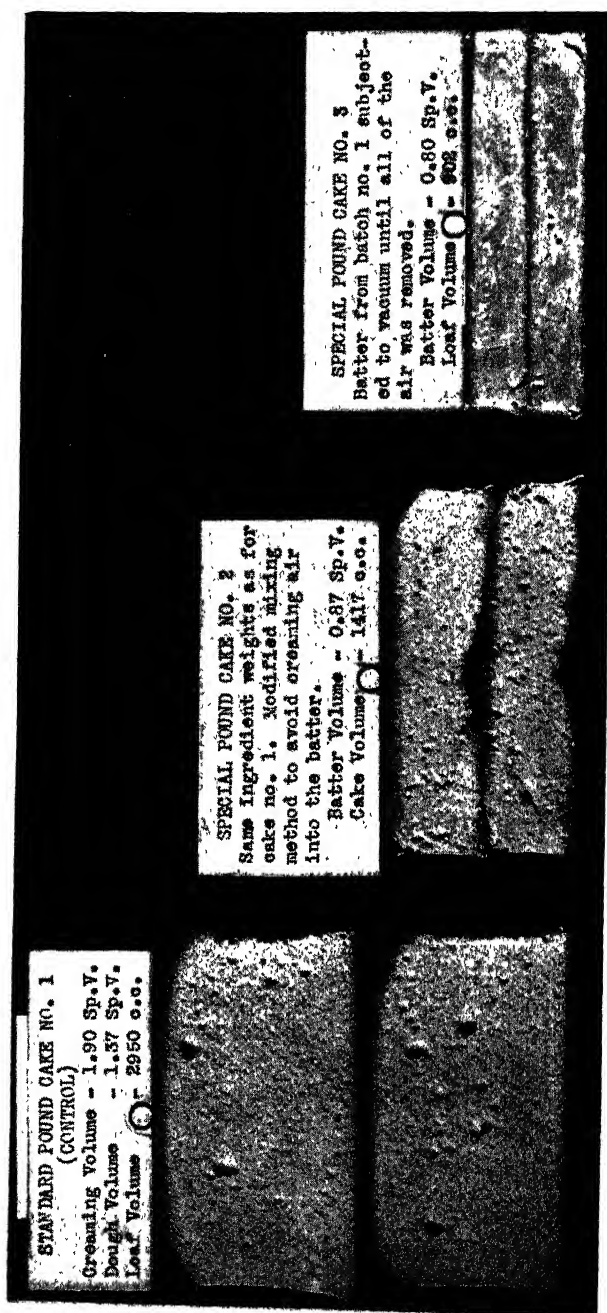


Fig. 1. The leavening action of air included in pound-cake batter.

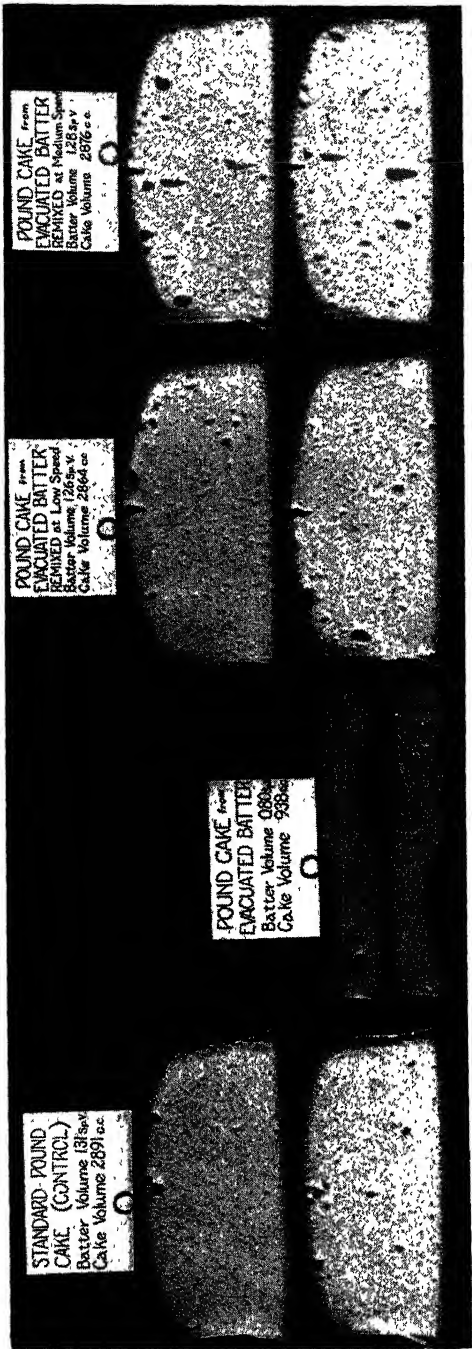


Fig 2. Air exhausted from pound-cake batter may be replaced by extended mixing.

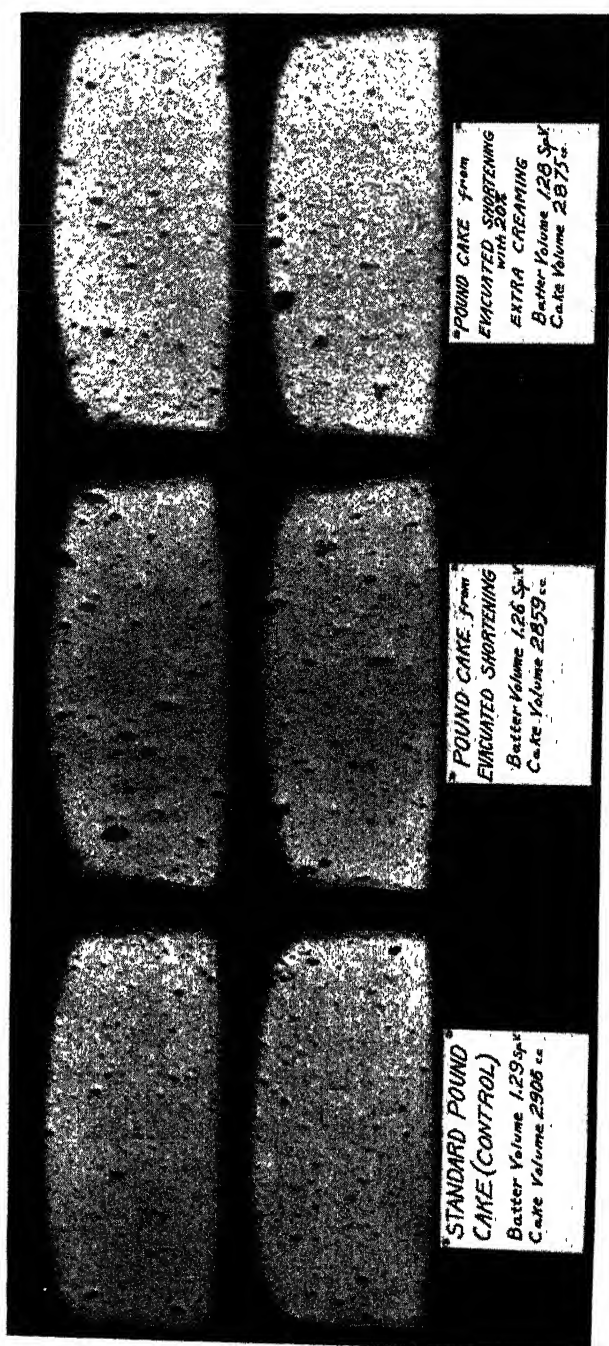


Fig. 3. Air introduced in the manufacture of shortening improves cakes and reduces creaming time

which is  $298.4^{\circ}$  A. The temperature of the finished cake as it left the oven was  $208^{\circ}$  F., which is  $370.8^{\circ}$  A. Dividing the lower absolute temperature into the higher, therefore, we find the air expansion factor is 1.242.

From the scaling weight, which was 1,135 g., and the specific volume of the batter (1.37), it is possible to calculate the actual volume of the batter scaled in each pan (1,555 c.c.). The volume of the finished cake was measured (2,950 c.c.). The difference between these two values represents the true expansion or volume increase (1,395 c.c.).

Because the specific volume of the air-free batter has been determined, it is also easy to show that the total volume of the air-free batter is 908 c.c. Thus the batter scaled in each pan contained 647 c.c. of air. Applying our expansion factor of 1.242, we can account for 803.6 c.c. of the total volume increase, as directly caused by the thermal expansion of the air. This figure is equivalent to 51.7% of the total increase.

The remainder of the volume increase must be due to the evaporation of liquid into the tiny air cells, and the subsequent expansion of this water vapor. This portion of the oven-expansion (48.3%) will be subject, of course, to a correction factor because of possible changes in volume of starch and gluten due to the action of moisture and oven heat.

For all practical purposes, it is safe to conclude that half of the volume increase is due to the air incorporated in the batter, the remainder being principally produced by moisture evaporating into the air cells and subsequently expanding.

Applying the same method to the data procured by the special method designed to prevent mixing air into the batter, we obtained figures indicating that about a fourth of the increase in volume was attributable to the thermal expansion of the air, the remainder being caused principally by the production and expansion of water vapor. It is interesting to observe, however, that when all of the air is evacuated so that there are no air cells into which the moisture can evaporate, the volume increase is zero.

### Summary

Half of the increase in volume of a lean pound cake containing no chemical leavening agents is due to the thermal expansion of air included in the batter.

When the air in a pound-cake batter is completely exhausted, a peculiar batter resembling custard cream is produced. When this batter is baked, the volume increase is zero.

It is possible to approach a batter containing no air by varying the mixing procedure; but even a small amount of air included in the batter results in a definite increase in volume during baking.

Air can be remixed into an evacuated batter and a reasonably good pound cake produced. The resulting cake, however, shows evidence of overmixing.

The incorporation of air in commercial shortenings improves the texture and volume of cake, and reduces the time required for proper creaming.

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## REPORT OF THE COMMITTEE ON TESTING RYE FLOUR

L. H. BAILEY, *Chairman*

Food Research Division, Bureau of Chemistry and Soils,  
U. S. Department of Agriculture, Washington, D. C.

(Read at the Annual Meeting, May 1937)

Five years ago a committee was appointed by the American Association of Cereal Chemists to develop a method that could be used to test the baking qualities of rye flours. This committee pointed out in its first report that its task was a large one with many complications. There was no standard for rye bread and no universal conception as to the ideal loaf of rye. There are at least five grades of rye flours with variations within these grades. Varying percentages of rye flour are mixed with clear flour to make the rye bread. Sometimes a mixture of two grades of rye flour is used as the rye component. Rye breads, like white breads, are made with sponge and dough as well as with straight doughs. "Swedish" rye is a large loaf, open grained, and sweet to the taste. It is made with sugar or molasses while pumpernickel has a very close, compact grain, with a sour taste when made with "sour" dough.

Out of this maze of variations the committee decided to limit its study to straight doughs made with yeast, salt, water, clear flour and the five principal grades of rye flours. At first both pan and hearth loaves were made, but later the pan loaves were discontinued. Neither "Swedish" rye nor pumpernickel was included in the studies.

While the proportions of rye flours of the different grades and the clear flour varied in the experiments, the total flour constituent was always kept at 200 g. The doughs were mixed in Hobart-Swanson mixers, when available. The total fermentation time, the proofing time, when to punch the doughs, and how many punches to make were all given consideration.

After the bread was made the trouble was to score it. There was no score card for rye bread, so the committee proceeded to develop one. After much study it was concluded that one score card could be used

with all grades of rye bread, the only condition being that breads from the same grade of rye flours be scored against each other. Thus the white rye breads should be scored by themselves, the medium ryes by themselves, and so on. The committee has been using numerical values for the different points being considered in scoring. On this point there is divided opinion among the members of the committee, some considering it preferable to use descriptive terms rather than numbers to express variations. Loaf volume and weight are given as actual values, and it would be a simple matter to use descriptive terms instead of arbitrary numerical values for the other scores.

Under crumb color, the committee had great difficulty in reaching an agreement in scoring collaborative bakings. In scoring white rye breads perhaps the highest score should be given to the one having the whitest crumb, while in scoring extra dark ryes the highest score would perhaps be given to the one that had the darkest colored crumb, but when it came to the medium ryes the task was not so easy.

To overcome this difficulty a color panel was prepared from rye breads of six different shades of color, ranging from that of white rye bread to that of extra dark rye bread. These breads were made by the method as now used by the committee. The bread was sliced and dried, then impregnated with collodion and mounted on a pasteboard panel. It was a simple matter to place a slice of the bread being tested alongside these breads on the panel and note which one most nearly matched in color. If the color fell between two of the pieces on the panel the fact was indicated by the use of the + and - signs; for example, 2+ to indicate a color between 2 and 3 on the panel. The colors of the breads on the panel were believed to cover the range that is likely to be met when using formulas and methods outlined by the committee. The formulas and methods follow:

#### METHOD FOR TESTING RYE FLOURS

##### Formulas for using six grades of rye flour

White and light rye bread		Cream and medium rye bread		Dark and extra dark rye bread	
	<i>Grams</i>		<i>Grams</i>		<i>Grams</i>
Clear flour	100	Clear flour	120	Clear flour	140
White or light rye flour	100	Cream or medium rye flour	80	Dark or extra dark rye flour	60
Yeast	4	Yeast	4	Yeast	4
Salt	4	Salt	4	Salt	4
Water (variable)	(120 c.c.)?	Water (variable)	(130 c.c.)?	Water (variable)	(135 c.c.)?
Mix for 1 minute in Hobart-Swanson mixer or equivalent.		Mix for 1 minute in Hobart-Swanson mixer or equivalent.		Mix for 1 minute in Hobart-Swanson mixer or equivalent.	
First punch after 75 minutes		First punch after 75 minutes		First punch after 75 minutes	
Second punch after 45 minutes		Second punch after 45 minutes		Mold after 45 minutes	
Mold after 30 minutes		Mold after 30 minutes		Proof to optimum	
Proof to optimum		Proof to optimum			

After proofing, give three slight slashes across the top of the doughs and place in oven. Bake at 230° C. for 30 to 35 minutes. Use plenty of steam in the oven or brush the loaves with water two or three times after the crust has formed. Cool on rack and score (preferably the following day).



These methods are the result of several years' study. They have been tested by the committee and have proved satisfactory for the purpose of testing the baking qualities of rye flours.

The score card would be satisfactory to the majority of the committee if descriptive terms were substituted for numerical scores for general appearance, crust color, grain, texture, odor and taste.

Scoring crumb color by means of the color panel has been used only this last year. While the collaborative results do not show the uniformity that was anticipated, it is the best scheme that has been tried so far.

For the collaborative work this year the same samples of white, medium, and dark rye flours, together with a clear flour, were furnished each of the collaborators. Formulas and methods previously worked out by the committee were used by the collaborators in testing these flours. The scoring was done according to the score card developed by the committee, using numerical scores for certain of the points under consideration and the color panel (furnished each collaborator) for scoring crumb color. The collaborative results are shown on the score card given.

SCORE CARD FOR COLLABORATIVE BAKING TESTS OF RYE FLOURS

Collaborator	Rye flour grade	Loaf volume	Loaf weight	General appearance (25)	Crust color (10)	Crumb color (by panel)	Crumb grain (20)	Crumb texture (20)	Odor and taste (25)	Remarks
A	White	840	260	23	9.5	1+	19	19	25	Creamy
B	"	840	270	23	10.0	1	19	20	23	
C	"	618	252	25	9.0	1-	17	17	24	
D	"	756	265	23	9.0	1+	19	18	21	
E	"	770	274	23	9.0	1	18	18	25	
A	Medium	855	267	23	9.5	2-	19	19	25	
B	"	925	272	25	10.0	1	19	20	23	
C	"	562	259	25	10.0	2+	18	19	24	
D	"	808	275	24	9.0	3+	18	18	23	
E	"	850	277	24	9.0	2-	18	19	25	
A	Dark	790	292	23	9.0	5-	19	19	25	
B	"	805	267	23	10.0	3	20	20	23	
C	"	438	271	25	10.0	4	19	19	24	
D	"	725	295	21	8.0	4	17	17	24	
E	"	645	297	23	9.0	4+	17	16	25	

The collaborators were the following:

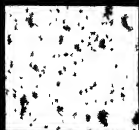
- T. W. Sanford, Eagle Roller Mill Company, New Ulm, Minnesota.
- G. Moen, General Mills, Inc., Minneapolis, Minnesota.
- I. O. Juvrud, The W. E. Long Company, Chicago, Illinois.
- J. T. Flohil, Pillsbury Flour Mills Company, Minneapolis, Minnesota.
- L. H. Bailey, Bureau of Chemistry and Soils, U. S. Dept. of Agriculture, Washington, D. C.

RYE BREAD PANEL

1



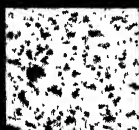
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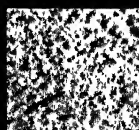
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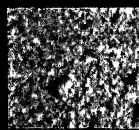
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5



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### Conclusions

The committee has now developed methods for testing rye flours of the different grades for their baking qualities.

It has developed a score card that is suitable for use with the different grades of rye flour. Descriptive terms may be substituted for numerical values if desired.

A color panel has been developed which shows variations in color between white rye bread and extra dark rye bread baked according to the methods prescribed.

It is recommended that:

The method here presented be adopted tentatively by this Association for the purpose of testing the baking qualities of rye flours.

(It is to be clearly understood that in developing this method no attempt has been made to standardize the commercial methods of making rye bread.)

It is further recommended that this committee, having completed its task, be discharged.

## REPORT OF THE 1936-37 COMMITTEE ON CAKE BAKING TESTS AND SELF-RISING FLOURS

L. D. WHITING, *Chairman*

Ballard and Ballard Co., Inc., Louisville, Kentucky

(Read at the Annual Meeting, May 1937)

The committee on Cake Baking Tests, consisting of H. R. Fisher, J. W. Montzheimer, W. C. Rohrbaugh, W. E. Stokes, Laura K. Track, and L. D. Whiting, centered its attention this year on the comparison of the A. A. C. C. Basic Cake-Baking Method (designated as Method 1) with a yellow formula using whole egg (designated as Method 2). The latter method was based upon the formula used by Cooley and Davies (*Cereal Chemistry* 13: 609-613, 1936).

Five soft-wheat flours varying widely in analysis, as shown in the following table, were baked by the five collaborators and scored according to the score card (*Cereal Chemistry* 8: 253).

Flour	No. 1	No. 2	No. 3	No. 4	No. 5
Moisture, %	12.8	11.6	11.0	11.4	12.1
Protein, <sup>1</sup> %	8.2	7.6	9.4	7.1	7.6
Ash, <sup>1</sup> %	0.42	0.35	0.35	0.32	0.35
pH	5.54	5.27	5.6	5.65	5.1

<sup>1</sup> Moisture basis 13.5%.

The results are shown in Tables I to III and Tables IA to IIIA.

TABLE I  
TOTAL AVERAGE SCORES

Collaborator	Flour no. 1		Flour no. 2		Flour no. 3		Flour no. 4		Flour no. 5	
	Method		Method		Method		Method		Method	
	1	2	1	2	1	2	1	2	1	2
A	61	76	68	75	67	80	45	64	53	75
B	57	48	77	78	73	70	59	54	86	96
C	82.3	83.5	87	90	84	84.5	77	78.5	89	91
D	65	56.5	87.5	89	80.25	67	71	65	97	94.5
E	79	75	87	85	75	71	72	69	89	90
Average	68.8	67.8	81.3	83.4	75.85	74.5	64.8	66.1	82.8	89.3

TABLE IA  
COMPOSITE RATING  
(By total average score)

	Method 1		Method 2	
	Score	Rating	Score	Rating
Flour 1	68.8	4	67.8	4
Flour 2	81.3	2	83.4	2
Flour 3	75.85	3	74.5	3
Flour 4	64.8	5	66.1	5
Flour 5	82.8	1	89.3	1

TABLE II  
VOLUME SCORES

Collaborator	Flour no. 1		Flour no. 2		Flour no. 3		Flour no. 4		Flour no. 5	
	Method		Method		Method		Method		Method	
	1	2	1	2	1	2	1	2	1	2
A	10	12	10	12	10	15	6	11	8	12
B	8.5	7	13.5	12	15	12	8	6	15	15
C	11.75	12.5	13.5	14	14	12.5	12	12.5	13.5	14
D	10	12.5	16	15	15.75	9	13	11.5	20	15.5
E	13	12	14	13	12	11	10	12	14	14
Average	10.65	11.2	13.4	13.2	13.35	11.9	9.8	10.6	14.1	14.1

TABLE IIA  
RATING BY AVERAGE VOLUME SCORE

	Method 1		Method 2	
	Score	Rating	Score	Rating
Flour 1	10.65	4	11.2	4
Flour 2	13.4	2	13.2	2
Flour 3	13.35	3	11.9	3
Flour 4	9.8	5	10.6	5
Flour 5	14.1	1	14.1	1

TABLE III  
TOTAL AVERAGE INTERNAL SCORES

Collabo- rator	Flour no. 1						Flour no. 2						Flour no. 3						Flour no. 4						Flour no. 5					
	Method 1			Method 2			Method 1			Method 2			Method 1			Method 2			Method 1			Method 2			Method 1			Method 2		
	Texture		Grain	Texture		Color	Texture		Grain	Texture		Color	Texture		Grain	Texture		Color	Texture		Grain	Texture		Color	Texture		Grain	Texture		Color
	16	15	10	22	20	10	20	16	12	23	18	12	20	15	8	25	20	8	16	10	5	21	18	5	16	10	11	22	18	11
A	14	13.5	7	11	8	10	21	16	12	20	20	12	20	14	11	20	14	11	14	15	8	13	15	8	21.5	21.5	14	28	24	14
B	23	21	13	26.5	21	11	26	21.5	13	28	21.5	13.5	23	24	12	28	21	11	22	20	10	23	21	10	26	22.5	14	28	22	14
C	18	15	10	13	10	12	23	22	12	26	22	14	21	18	11	18	15	14	20	16	10	20	12	13	20	20	14	30	22	15
D	24	18	12	20	20	12	26	22	13	25	22	13	22	19	11	19	19	12	22	17	11	19	18	12	26	22	14	28	22	13
E	19	16.5	10.4	18.5	13.8	11	23.2	19.5	12.4	24.4	20.7	12.9	21.2	18	10.6	22	17.8	11.2	18.8	15.6	8.8	19.2	16.8	9.6	23.7	19.2	13.4	27.2	21.6	13.4
Average																														
Av. total	45.9			45.3			55.1			58.0			49.8			51.0			43.2			45.6			56.3			62.2		

TABLE IIIA  
RATING BY INTERNAL SCORE

	Method 1						Method 2					
	Texture		Grain	Texture		Color	Texture		Grain	Color		Rating
	1	2	3	4	5	6	1	2	3	4	5	6
Flour 1	4	4	4	4	45.9	4	5	2	5	4	45.3	5
Flour 2	2	2	2	2	58.1	2	2	2	2	2	58.0	2
Flour 3	3	3	3	3	49.8	3	3	3	3	3	49.6	3
Flour 4	6	6	6	6	43.2	6	4	4	4	4	45.6	4
Flour 5	1	2	2	1	56.3	1	1	1	1	1	62.2	1

### Summary

The total average score figures for cakes made by the two methods very closely approximated each other. The effect of method of mixing upon general scoring was very little in comparison with the differences brought out in the cakes by varying the flour.

The general effect of the total average score (Table I) for the series of cakes made by the five collaborators in this test was to rate the methods as the same. The flour ratings ranked the same for both Methods 1 and 2 (Table IA).

When the volume score (Table II) was viewed separately, the difference between methods was little but the differences from flour sample to flour sample became evident. Here the ratings for the flours were the same under the two methods and took the same ranking as when rated by total average score (Tables IA and IIA).

From the scores on internal characteristics, it would seem that Method 1 is perhaps a more severe color test, since all the flours tested rated a better score for color under Method 2 than under Method 1. The figures for texture and grain again showed greater differences from flour sample to flour sample than between methods, although when these were gathered into rating form the difference between the methods was reflected in the rating order; Method 1, in texture, gave fourth place to flour sample No. 1, and fifth place to flour sample No. 4, while Method 2 reversed these standings. The rankings of the other flours were the same under both methods.

The grain rating under Method 1 gave first place to flour No. 2 while flour No. 5, which ranked first on every other point, took second place. The ratings of the flours No. 1 and No. 4 were reversed by method, Method 1 giving flour No. 1 fourth place and flour No. 4 fifth place; while Method 2 gave flour No. 1 fifth place and flour No. 4 fourth place. The color ratings for flour ranked the same under both methods although the actual score figures for color were higher under Method 2.

Both the scoring figures and the rating figures, viewed compositely and individually, indicate that there is little choice between the two methods for the purpose of testing soft-wheat flours by baking, except for the value of the color test, which is reflected more sharply by Method 1.

The committee gratefully acknowledges the valuable assistance of Laura K. Track in compiling the results of these tests.

## REPORT OF THE SUB-COMMITTEE ON METHODS OF TESTING CAKE FLOUR, 1937-38

J. W. MONTZHEIMER, *Chairman*

Centennial Flouring Mills Co., Spokane, Washington

(Read at the Annual Meeting, May 1938)

This year's project was devised especially to compare the results obtained in the laboratories of different collaborators using the A. A. C. C. formula but varying the mixing methods.

Three flours were furnished to this year's committee, all of them commercial cake flours milled in various sections of the country. Each was made from different types of wheat and was considered commercially satisfactory. The approximate protein contents of these flours were 9, 8, and 7%, respectively. All of the ingredients used in this year's work, with the exception of the frozen egg albumen, were furnished to the committee by the chairman. Special care was taken to have all ingredients uniform and of the highest quality. Sugar of an extra fine granulation was chosen, this being sold to the bakers under the grade of Baker's Special. The following five mixing procedures and formulas were used, but the proportions of ingredients were all the same, using the tentative A. A. C. C. formula, Cereal Laboratory Methods, third edition, pages 78-79:

Formula 1: Tentative A. A. C. C. formula using frozen egg albumen.

Formula 2: Tentative A. A. C. C. formula using domestic dehydrated egg albumen.

Formula 3: Tentative A. A. C. C. formula. Cream the sugar and shortening two minutes at second speed. Add frozen-egg albumen slowly to sugar-shortening mix and continue creaming for three minutes. Sift flour, salt, soda, and add to batter alternately with milk, mixing at slow speed for four minutes. Add cream of tartar and mix two minutes.

Formula 4: Tentative A. A. C. C. formula using all dried ingredients. Sift sugar, flour, milk, egg albumen, soda, and salt together three times. Place in mixer. Add shortening; add 100 c.c. water and mix at second speed for two minutes. Add 100 c.c. water and mix three minutes. Add 80 c.c. water and mix four minutes. Then add cream of tartar and mix two minutes.

Formula 5: Tentative A. A. C. C. formula. Place flour, sugar, shortening, soda, and salt together with 150 c.c. milk in mixer and



mix at second speed four minutes. Add 130 c.c. milk and 80 c.c. frozen egg albumen gradually and mix at slow speed for five minutes. Add cream of tartar and mix two minutes.

The three samples of flour were tested by the committee for moisture, protein, ash, pH, viscosity, and flour granulation. Cakes were graded according to present A. A. C. C. official score card. Cake batters were tested for specific volume according to the method outlined by Dunn (*Cereal Chemistry* 14: 783). Cake volume was measured in cubic centimeters. Weight of cakes was measured in grams, and specific volumes were computed. Each collaborator reported altitude of laboratory and barometric pressure at time of baking. Type of equipment used in each of the various laboratories was noted.

TABLE I  
AVERAGE OF ANALYTICAL RESULTS OF DIFFERENT COLLABORATORS

	Flours		
	A	B	C
Moisture, %	11.5	11.9	11.7
Protein, basis 15% moisture	9.07	7.22	8.21
Ash, basis 15% moisture	.407	.318	.384
pH	5.36	5.00	5.26

### Discussion of Results

Variation of moisture reports was caused by drying out of samples during transit and before testing in the laboratories.

Checks between collaborators on well established methods such as ash, protein, and pH were reasonably close.

Only three collaborators reported on viscosity. Results were not concordant.

Granulation tests have not been sufficiently standardized as to method and equipment to obtain valuable results in collaborative work.

Differences in cake character were caused, in this year's work, by difference in pan sizes. Specifications in the Book of Methods do not state whether the figures given for length and breadth of pan apply to top or bottom measurements. Because of the flare in most pans there was a variation in pan size; some collaborators applied their specifications to the top and others to the bottom of the pan.

Cakes were scored differently by different operators, some scoring very severely while others were more lenient.

Comprehensive analysis of reports and summary of results made by Mrs. Laura Track showed that the averages of all of the collaborators for all five formulas gave the flour the same order of rating.

### Comments of Collaborators

All of the committee members preferred frozen egg albumen to the powdered egg albumen furnished for this year's work. Six of the committee members preferred the mixing method followed in Formula 5 because it produces a batter of smooth consistency. All collaborators voted to recommend that next year's committee compare commercial-type formulas with our present method of testing.

A majority of the committee were favorable to the specific volume test for testing cake batter because it was valuable in predicting the volume of the finished cake. Several of the committee felt that the test was not as valuable for baking-powder-leavened cakes as it would be in pound or sponge cakes which depend on creaming volume or incorporated air for lightness.

### Recommendations for Next Year's Committee

1. That no changes be made on the present A. A. C. C. formula until further studies are completed.
2. That the following dimensions be adopted for the loaf pan: top,  $8'' \times 4''$ ; bottom,  $7\frac{1}{2}'' \times 3\frac{1}{4}''$ ; depth,  $2\frac{1}{2}''$ .
3. That the following dimensions be adopted as official for round layer cake pan:  $8'' \times 1\frac{1}{2}''$ .
4. That mixing methods be controlled by stating the number of revolutions of the paddle, rather than the mixing time. (There is a variation in speed of the mixers used by different committee members.)

### Recommendations for Future Study of the Cake Committee

1. That type photographs of both loaf and layer cakes be made and circulated among committee members for criticism, in order to set up definite photographic standards in grading the interior and exterior properties of cakes.

2. Since this year's project shows many differences in baking results, which may be caused by variations of baking conditions, the committee recommends that a close record be kept of: (1) baking time, (2) of temperature in the oven 2 inches directly over the cake pan—this can be accomplished by suspending a thermometer in the oven two inches above the baking cake, (3) baking losses, with the idea of bringing losses among collaborators to the same magnitude. (This year's committee showed baking losses from 25 to 70 g. for cakes baked in different laboratories with the same ingredients and formula and supposedly the same baking conditions.)

3. Standard cake liners should be used by the committee.
4. Future committees should report pH on finished cake.

5. Since there is a demand by certain chemists for investigation of commercial-type formulas, it is recommended that a study of commercial formulas, which have been assembled by this year's committee, be carefully made during the first part of the year. One or more of these formulas should be set up in the laboratories of the various collaborators, so that a comparison may be made on routine work between the new formula being investigated, the present A. A. C. C. formula, and the formula habitually used for testing purposes in the respective laboratories. It is suggested that the comparison be made under laboratory conditions, at least once each week for the first three months, and after the formula has been studied in this manner it be corrected and standardized for consideration of the committee as a whole. Any formulas receiving especially favorable rating could then be used for collaborative work during the remainder of the year. In this manner the value of commercial types of cake formulas for testing purposes could be compared with that of our present method and the committee would then be in a position to recommend either the continuance of the A. A. C. C. method or a good type of cake formula as a substitute for it.

#### Membership

J. W. Montzheimer,	J. R. Davies	O. E. Stamberg
Chairman	E. P. Walker	F. J. Coughlin
W. E. Stokes	W. C. Rohrbaugh	F. D. Machon
R. W. Mitchell	H. R. Fisher	L. D. Whiting

The committee wishes to acknowledge the assistance of Donald Wade of Mr. Coughlin's staff and of Mrs. Laura Track of Dr. Stokes's staff.

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### SUPPLEMENT TO REPORT OF 1937-38 COMMITTEE ON TESTING CAKE FLOUR

W. E. STOKES and LAURA K. TRACK <sup>1</sup>

Standard Brands, Inc.

Royal Baking Powder Factory, Brooklyn, N. Y.

Ratings for baking qualities of the several flours were based upon the score figures allocated by the individual collaborators. Separate ratings were given for external score, internal score, total score, and volume. Ratings were arrived at by giving "first place" to highest score, "second place" to second-highest score, and "third place" for low score. The ratings thus obtained for volume, external and internal and total scores, were ascertained by totaling the number of ratings in each place for each flour, thus setting up a final place rating. For example under Formula 1, one of the collaborators scored the flours for external baking characteristics as follows:

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<sup>1</sup> Report of 1937-38 sub-committee on Testing Soft-Wheat Flours.

Flour A	25	Rating
Flour B	21	1
Flour C	21	2
For internal, as follows:		
Flour A	52	Rating
Flour B	56	2
Flour C	51	1
For total score, as follows:		
Flour A	77	Rating
Flour B	77	1
Flour C	72	2
For volume, as follows:		
Flour A	768	Rating
Flour B	702	1
Flour C	727	3

Thus the total of "first" ratings for flour A was 3, and the number of "second" ratings, 1. Flour B totaled two "first" ratings, one "second," and one "third." Flour C showed no "first" rating, three "second," and one "third" place.

Flour A, having scored the greatest number of "first" ratings and no "third" rating, was given a final place rating of one. Flour B with two "first" ratings, one "second," and one "third," scored a final-place rating of two. Flour C showed out in a final "third" place rating with no "first," three "seconds" and one "third" place rating.

The final composite place ratings were obtained by totaling, as in the foregoing, the "final place ratings" by individual collaborators' scores. Of the ten collaborators, three gave first ratings to flour A, six first place to flour B, and one gave first rating to flour C.

Five of the collaborators by their scores rated flour A for second place, four gave flour B second place, and two gave second place to flour C. Final consideration of these place ratings brought the final place ratings:

First place	Flour B
Second place	Flour A
Third place	Flour C

This plan was carried through for the three flours, five formulas, ten collaborators, arriving at the final composite rating under all formulas as:

Flour A	2
Flour B	1
Flour C	3

Place ratings given the flour samples on an average basis rated in the same order under formulas 3, 4 and 5, as when computed on the composite basis, but under formulas 1 and 2, the final *average* place ratings were:

Flour A	1
Flour B	2
Flour C	3



## FINAL PLACE RATINGS

ACCORDED THREE FLOUR SAMPLES UNDER FIVE FORMULAS BY COLLABORATORS

Collaborators	Formula 1			Formula 2			Formula 3			Formula 4			Formula 5		
	Flours			Flours			Flours			Flours			Flours		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
E. P. Walker	1	2	3	2	1	3	2	1	3	2	1	3	2	1	3
W. C. Rohrbaugh	2	1	3	—	1	2	3	1	2	2	1	3	3	1	2
J. W. Montzheimer	2	1	3	3	1	2	1	2	3	1	3	2	1	2	3
R. W. Mitchell	3	1	2	2	1	3	3	1	2	2	1	2	2	1	3
Donald Wade	1	2	2	1	2	2	2	1	3	1	1	2	2	1	3
Olaf Stamberg	1	2	3	2	1	3	2	1	3	2	1	3	2	1	3
W. E. Stokes	2	1	3	2	1	3	2	1	3	3	1	2	2	3	1
F. D. Machon	2	1	3	2	1	3	3	1	2	3	2	1	3	1	2
H. R. Fisher	3	2	1	1	2	2	2	1	3	2	1	3	2	1	3
L. D. Whiting	2	1	3	1	3	2	3	1	2	1	2	3	1	1	2
Number of place ratings:															
First	3	6	1	3	7	0	1	9	0	3	7	1	2	8	1
Second	5	4	2	5	2	5	5	1	4	5	2	4	6	1	3
Third	2	0	7	1	1	5	4	0	6	2	1	5	2	1	6
Final composite place rating	2	1	3	2	1	3	2	1	3	2	1	3	2	1	3

## REPORT OF 1937-38 SUB-COMMITTEE ON METHODS OF TESTING SELF-RISING FLOURS

O. E. GOOKINS

Quaker Oats Co., St. Joseph, Mo.

(Read at the Annual Meeting, May 1938)

The personnel of the 1937-38 sub-committee on Methods of Testing Self-Rising Flours was as follows: R. A. Barackman, L. E. Leatherock, Harold McGhee, Elizabeth McKim, H. V. Moss, Grant W. Percy, C. C. Walker, and O. E. Gookins, chairman. Five members of this committee are connected with milling concerns and three with chemical companies supplying self-rising ingredients.

A general summary of work done by previous committees on testing self-rising flours was prepared and presented to the members of the committee. A questionnaire was also sent out covering various items suggested by former committees, and tentative subjects for study this year. The replies brought out the following points:

1. Everyone approved continuing with the 1935 biscuit recipe (flour 15% moisture basis, 227.7 g.; soda 3.4 g.; mono-calcium phosphate 4.3 g.; salt 4.6 g.; hydrogenated shortening 30 g.); however, two members suggested less shortening for soft flours.

2. Five approved the method of mixing (as given by the 1933-34 committee, *Cereal Chemistry* 12: 162). Suggested improvements were:

- Make the method faster and more usable in practice.
- Obtain better fat distribution.
- Omit one fold and one roll of dough.
- Study further the variables.

3. Definite specifications for a possible flour standard were given in only one instance, the general feeling being that we do not have enough experience to make recommendations. Ash, protein, pH, color, absorption, viscosity, moisture, and granulation were all suggested as factors to be considered. One suggestion was to have at least three hard and three soft wheat flour standards.

4. No one had a satisfactory method for flour color determination that could be duplicated in other laboratories.

The program of work taken up by this year's committee was in the nature of a study of various items affecting baking conditions and the scoring system. The work was done as individual assignments, with committee collaboration on some of the problems.

R. A. Barackman reported on the effect of one fold, two rolls of dough vs. two folds, three rolls. Using a soft wheat patent flour at 64% absorption, and with A. A. C. C. formula and procedure, the following data (average of three trials) were obtained:

	Wt. dough	Oven loss	Biscuit vol.	Specific vol. <i>c.c. biscuits/ g. dough</i>
	<i>g.</i>	<i>%</i>	<i>c.c.</i>	
1 fold, 2 rolls	156	16.0	348	2.23
2 folds, 3 rolls	173	16.2	373	2.16

It will be noted that the one-fold, two-roll method allows 10% lighter doughs to be cut, 6.7% less volume of biscuits, and 3% lighter biscuits in terms of specific volume. The grain of the biscuits made by the one-fold, two-roll method was somewhat easier to judge, but substantially the same score would be assigned to biscuits from both methods.

Barackman also reported on the variations in biscuit bakings due to ovens. His data for electric vs. gas-oven baking (average of five replicate bakes in each) follow:

Oven	Bake temp.	Wt. of dough	Oven loss	Biscuit vol.	Spec. vol.	Crust score
		<i>g.</i>		<i>c.c.</i>	<i>c.c./g.</i>	
Electric	475° F.	161	14.7%	353	2.194	9
Gas	475° F.	160	16.7%	352	2.202	10

Both ovens had automatic temperature controls, which were checked by thermometer readings. Gas or electric oven baking causes no appreciable difference in volume of baked product. The biscuits baked in an electric oven had a somewhat paler crust color compared to those in the gas oven. A greater oven loss was obtained with gas. No other differences in points of scoring biscuits were observed. These findings agree with the 1934-35 committee report in regard to oven losses, but not in the matter of volume. Their report stated that "Higher oven losses were accompanied by greater specific volumes." This discrepancy is evidently due to the fact that the former conclusion was based on the data of several collaborators who baked in different laboratories with one type of oven only. The present report has to do with the careful comparisons by one operator within a single laboratory.

C. C. Walker reported on a study of water absorption as related to biscuit baking. Using a short patent, soft-wheat flour, which showed a farinograph absorption of 56.3% at 15% moisture, and baking by A. A. C. C. method, the following specific volumes were obtained for varied amounts of water:

Absorption, % (basis 15% moisture)	No. replicate bakes	Average spec. vol.	Comments
56.3	1	1.97	Dry
57.0	1	1.97	Dry
60.0	6	2.02	Good
61.0	2	1.87	Good
61.5	2	1.88	Good
62.0	3	1.87	Slack
63.0	5	1.90	Slack
65.0	1	1.81	Very slack

The specific volumes did not vary much with different absorptions. The color and texture were about the same, except in the very dry doughs where the inside of the biscuit was too compact. Optimum absorptions, considerably higher than the farinograph absorption, confirmed results obtained by the 1934-35 committee on Methods of Testing Self-Rising Flours.

Elizabeth McKim and H. V. Moss had as their problem the study of definition and evaluation of various items on the score card. A paper is to be presented by them on this subject.

Grant W. Pearcy continued his work of last year on preparation of permanent color standards for biscuits. His report on color determinations will also be given separately.

One suggestion made to the committee was to use a sheet of metal lath (plaster lath) in the oven under the baking pan. This seemed to improve the evenness of the bake.



This concludes the report of this year's work, which may be summed up as follows:

1. One-fold, two-roll procedure is preferable to two-fold, three-roll method.
2. There is little difference between gas and electric ovens as affecting biscuit score, except the crust color.
3. Absorption variation of small percentages is not particularly critical in biscuit scoring.
4. Tentative definitions of score-card terms are outlined.
5. Series of photographs, representing a range of textures, are proposed for reference standards.
6. A method of preparing semi-permanent color standards for biscuit scoring is given along with collaborative tests.

Items 4 and 5 are covered in papers by McKim and Moss, and Item 6 by Pearcy.

Next year's committee may find it advisable to do more work on the actual technique of baking, and to try out, by collaborative baking, the definitions and texture and color scoring methods presented in this year's committee work. It is also suggested that work be done on a supplementary formula for indicating the best color possible in lower grades of flour.

The percentages of phosphate and soda now in use are those recommended by the 1931-32 committee. The ratio of soda to mono-calcium phosphate is 0.791. This is satisfactory for patents, but for lower grades of flour the biscuit color is unduly dark and creamy. A ratio of soda to phosphate in the range of 0.73 to 0.75 produces much different results on flours of above types, and is, perhaps, more in line with commercial practice.

Another supplementary test which might merit some investigation is flour granulation as a means of further classifying flours for self-rising purposes.

#### Previous Reports Cited

Walter, H. G.

- 1935 Report of the 1933-34 committee on methods of testing self-rising flours. *Cereal Chem.* 12: 162.
- 1936 Report of 1934-35 committee on methods of testing self-rising flours. *Cereal Chem.* 13: 722-723.

# STUDY OF DEFINITION AND EVALUATION OF VARIOUS ITEMS ON SCORE CARD <sup>1</sup>

ELIZABETH MCKIM and H. V. MOSS

Monsanto Chemical Co., St. Louis, Mo.

(Read at the Annual Meeting, May 1938)

As a part of the activities of the sub-committee on Methods of Testing Self-Rising Flours the assignment of defining and evaluating the five score items on the A. A. C. C. biscuit score card was allocated to two of the collaborators. In an endeavor to clarify the meanings of the various score items, the collaborators enlisted the aid of the other committee members and circulated a questionnaire requesting individual definitions and methods now employed by the committee members for evaluation of scores. From the replies, the following consensus on definitions and methods has been reached:

## Grain

*Definition:* An index of the physical structure of the biscuit crumb with reference to (a) the *size, shape and homogeneity of cells*, and (b) the *thickness of the cell wall*.

*Method of evaluating:* Judging by visual observation of horizontal or vertical sections of cold biscuits with or without reference to a control.

## Tenderness

*Definition:* A measure of the resistance to shear and compression of the biscuit crust and crumb.

*Method of evaluating:* Personal judgment of the feel of a biscuit when broken, compressed, or eaten with or without reference to control.

## Flavor

*Definition:* The quality of a flour which affects its smell and the taste and aroma of a biscuit baked from it.

*Evaluation:* Organoleptic comparisons with reference to a control involving: (a) the smell of a flour at room temperature or when mixed in hot water, and (b) the smell and taste of hot or cold biscuits. Scoring to be made with consideration of the following points given by R. A. Barackman in the 1933-34 self-rising flour committee report

<sup>1</sup> Report of 1937-38 sub-committee on Testing Self-Rising Flours.

(Cereal Chemistry 12: 164); the extent of mark-down to be dependent upon the judgment of the technician with reference to a sound, fresh, properly leavened standard flour and the numerical score to be accompanied with suitable descriptive terms:

	<i>Desirable</i>	<i>Undesirable</i>
Flour condition	Sweet	Rancid, musty
Flour quality	Sweet	Wheaty, starchy
Leavening	Neutral	Acid or sour, alkaline or soapy
Salt	Pleasing	Salty, flat
Eating quality	Good or chewy	Doughy, dry and crumbly

### Crumb Color

*Definition:* A comparative expression of the color of the cut surface of the biscuit crumb.

*Evaluation:* Visual comparison with reference to one or more controls.

### Volume

*Definition:* The average specific volume of the baked biscuit on a basis of the weight of dough with reference to a standard.

*Evaluation:* Determination of the volume of biscuits by seed displacement divided by the weight of dough; this calculated to a ratio of 2.00 as standard and weighted to 40 on the score card, *viz:*

$$\frac{\text{c.c. biscuit}}{\text{g. dough}} / 2.00 \times 40 = \text{volume.}$$

### Discussion

*Grain:* There is good agreement on definition, with uniform stress on characteristics of the cells and cell walls. Judging grain depended in many cases wholly on the conception of the technician without reference to a control. The need of standardization in judging grain is shown by comments of the collaborators suggesting preparation of reference standards embodying:

- (a) Photographic plates
- (b) Ink prints
- (c) Preserved biscuit sections or physical measurements including:
- (d) Moisture absorption
- (e) Sand penetration

All of these have been suggested or used with more or less success in bread and cake work.

*Tenderness:* Agreement on definition is good. Method of evaluation depends in every case on the response of a biscuit to breakage or

compression of the crumb by hand, or to the feel of the biscuit when eaten, both the crust and crumb being considered. In no case is "tenderness" being gauged mechanically. Some little experience in this collaborator's laboratory on a means of judging tenderness mechanically indicated that a reliable measure depended upon a large number of determinations of carefully prepared biscuit sections, and made the particular method tried impractical as a routine procedure. In spite of the evident uncertainty of the method now generally practiced, it appears to be the only practical one available; reference to a control is deemed desirable as some basis of comparison.

*Flavor:* The A. A. C. C. score card divides the flavor score into "flour quality" and "eating quality" as distinct from tenderness. Attention is called to the characteristics of "eating quality" in the tabulation of points to consider in judging flavor. Clarification of this point in the minds of the committee members is needed and consideration should be given to whether "eating quality" should not be included as a tenderness characteristic rather than a property of flavor.

Judging the smell of the flour prior to baking is deemed necessary since foreign odors or contamination are frequently encountered during mixing and the consumer is affected by this observation.

*Color:* The need for standardization of color score has been recognized by previous committees; this is brought out again by replies to the questionnaire which suggest as controls:

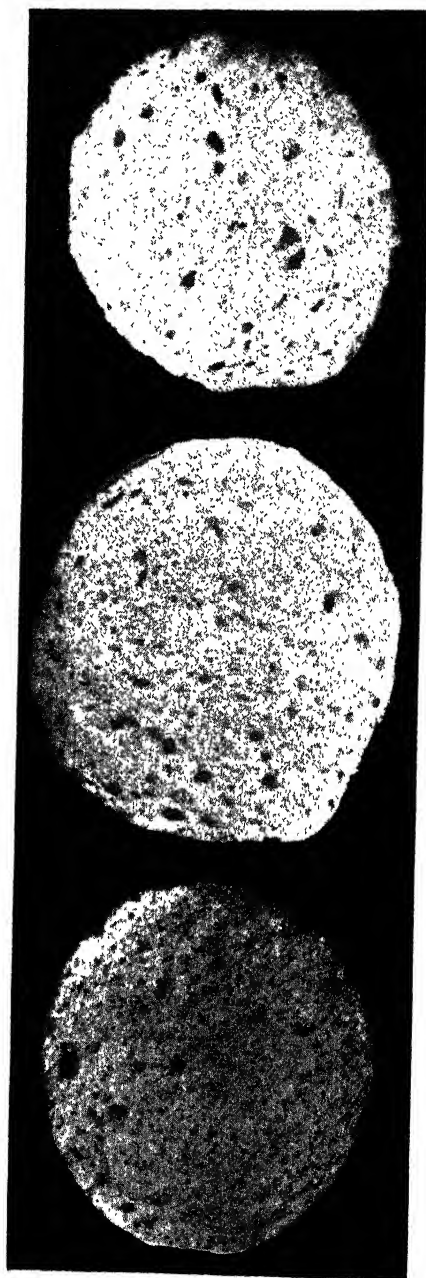
- (a) Biscuits baked concurrently with the unknown from one or more flours varying within known limits in color characteristics
- (b) Reference to color disks, or
- (c) Use of preserved biscuit sections

This last method was investigated in a preliminary way by the 1936-37 committee and because of favorable indications work on the preparation of suitable preserved standards has been continued this year by Grant Percy.

A value of 20 is attributed to crumb color in the A. A. C. C. biscuit score card and several of the committee members have pointed out that from a commercial viewpoint, this characteristic is of greater relative importance. From the experience of these collaborators, this appears to be a justified criticism, and it is suggested that consideration be given to revision of the A. A. C. C. biscuit score, with a view to increasing the importance of the crumb color score.

*Volume:* This is the only item in the biscuit score card which is reasonably well standardized. In a series of tests made by the 1933-34 committee on self-rising flours it was shown that three collaborators agreed within 2.3% on individual specific-volume determinations after

PLATE I  
Typical biscuit textures from flour types



"Close; Bready"  
Hard Wheat  
70% Patent

"Standard"  
Soft Wheat  
40% Patent

"Coarse"  
Soft Wheat  
2nd Clear

PLATE II  
Typical biscuit textures from leavening variations

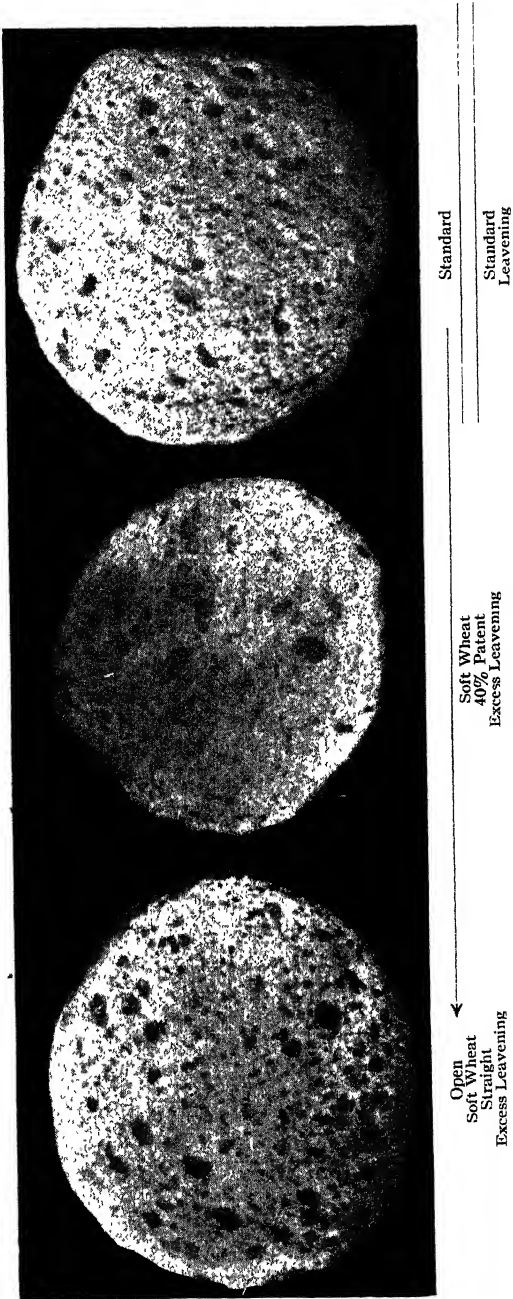


PLATE II—Continued

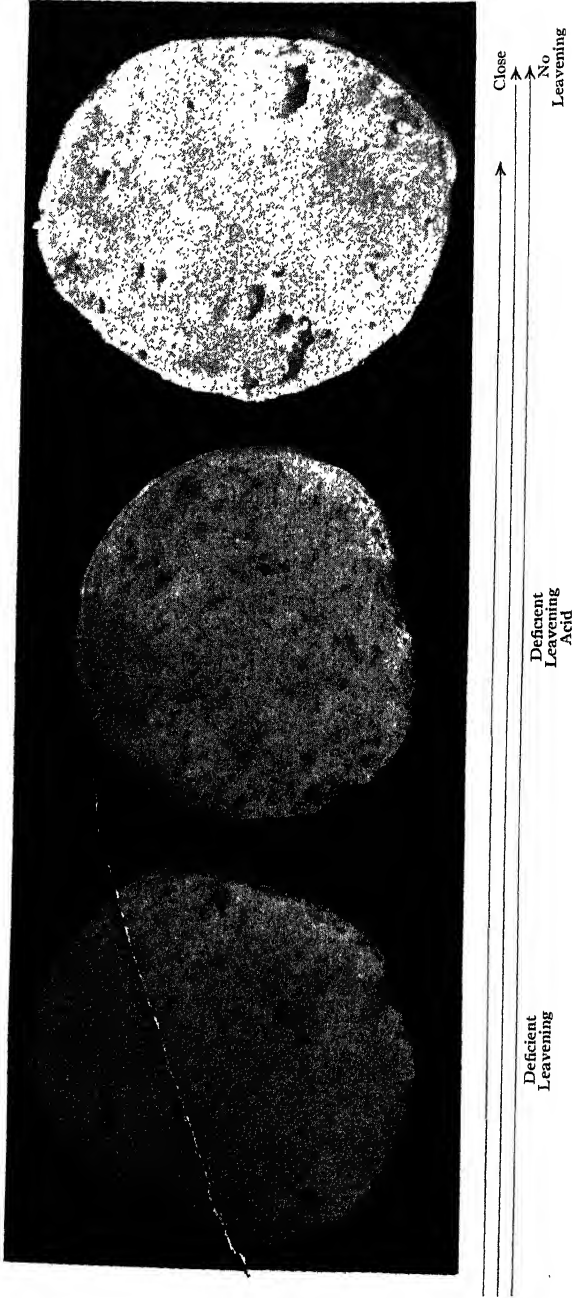


PLATE III  
Proposed standard texture types

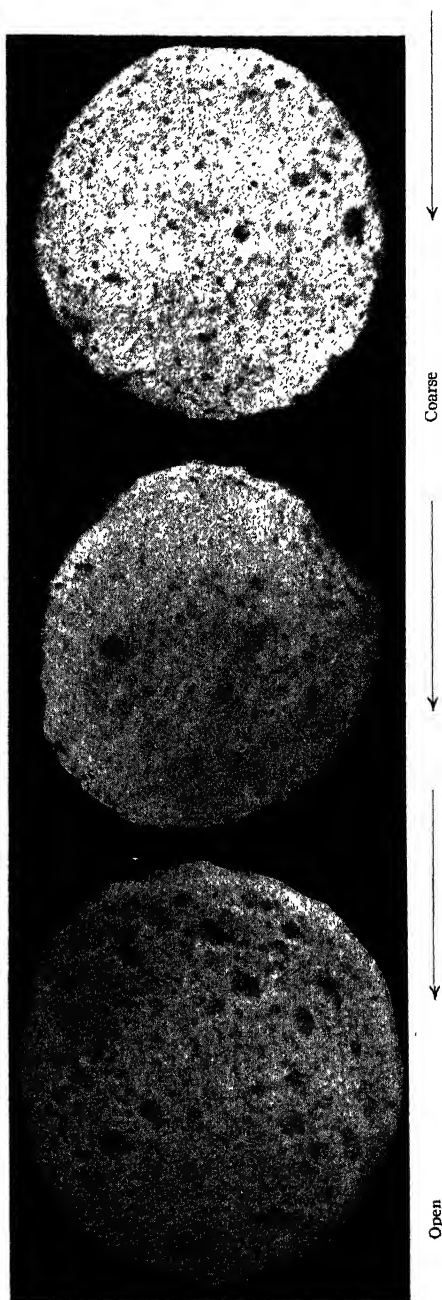
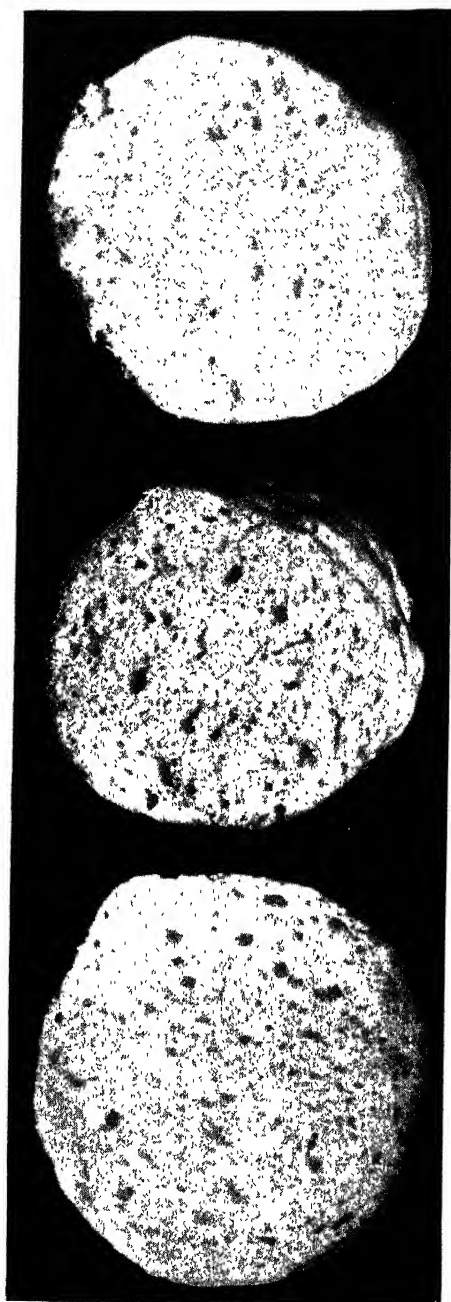




PLATE III—Continued



Heavy

Standard

PLATE III—Continued



preliminary calibration of measuring apparatus by means of wooden biscuit dummies of known volume.

The value of 40 now allotted to "volume" on the A. A. C. C. biscuit score appears to be given a disproportionate credit. Whereas volume is recognized to be a good relative index of lightness and desirable texture, it is also recognized that in many of the real biscuit-eating territories, a large, high biscuit is not necessarily a criterion of quality and furthermore it has been the experience of these collaborators that "volume" is subject to less variation with changes in self-rising flour quality than some of the other factors in the score. It is therefore suggested that consideration be given to revising the "volume" score downward, giving preference to color, grain, and tenderness in the order named.

In carrying out the assignment given us by the chairman of the Sub-Committee on Methods of Testing Self-Rising Flours, we have endeavored to prepare a set of photographic plates designed to illustrate typical biscuit textures with a view to establishing ready reference standards for judging biscuit grain or texture. The plates are self-explanatory and represent a suggestion of what may be accomplished. It will be observed that the pictures made so far are of horizontal biscuit sections; it is acknowledged that vertical sections are equally desirable and continuation of the work contemplates making pictures of these as well.

Plates I and II represent textures encountered with flour types and leavening variations respectively. Plate III is a composite of Plates I and II arranged to show trends in grain change. An increasing heaviness and closeness of grain is illustrated by the biscuits to the right, while the biscuits to the left of the standard illustrate increasing "coarseness" and irregularity of "open" texture.

It is a question whether normal judges of biscuit grain are able to recognize the differences in the pictures, with which we have become thoroughly familiar after numerous attempts at portraying photographically what we were able to observe in the "live" biscuit. As the next step in the development of standard grain plates it is proposed to supply the committee members with shuffled sets of pictures, asking that they arrange them in the order of their conception of quality and that they assign a numerical score to each biscuit. It is hoped that by this procedure a set of typical standard grain or texture plates may be established in which the range of the grain score will be shown.

# CONVENIENT CRUMB COLOR STANDARDS FOR SELF-RISING FLOURS <sup>1</sup>

GRANT W. PEARCY

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H. W. PUTNAM

Igleheart Brothers, Incorporated, Evansville, Indiana

(Read at the Annual Meeting, May 1938)

An unpublished progress report on the preparation and collaborative testing of convenient crumb-color standards for self-rising flours was read at the annual meeting, 1937, as a sub-project of the committee on Cake Baking Tests and Self-Rising Flours. That report indicated that reasonable agreement seemed possible between collaborators who were measuring biscuit crumb color in terms of a somewhat absolute scale instead of in descriptive terms or by means of indefinite numerical ratings.

Work this year was planned largely to deal with several matters proposed at that meeting, and included: (1) improvement of the standard board, (2) the further collection of collaborative data, (3) determination of changes during the year in the standards originally prepared, and (4) determination of the effect of sunlight on the collodion preserved biscuit slices. Work done previously was reviewed.<sup>2</sup> Bailey's method for scoring rye bread, privately communicated, was adapted to the preparation of standards for biscuit crumb color.

## Preparation of the "Color Charts"

The "color charts" consist essentially of black cards, with two closely parallel rows of windows, fastened to a piece of colorless clear thin glass. To the back of one row are fastened thin dried slices of biscuit crumb preserved in dilute collodion. The unknown is viewed through the openings in the adjacent row and is identified by the number of the standard matched.

Seven flour blends, selected to give an evenly spaced range of crumb colors, were baked, with every precaution taken to prevent streaks.

<sup>1</sup> Sub-committee report, 1937-38 Committee on Testing Self-Rising Flours.

<sup>2</sup> L. H. Bailey, Report of the committee on testing rye flour, *Cereal Chem.* 13, 770-772, 1936. J. C. Baker, H. K. Parker, and F. B. Freese, The measurement of color in flour and bread by means of Maxwell discs, II, *Cereal Chem.* 12, 17-24, 1935. Emily Grewe, W. K. Marshall, and C. G. Harrel, A method of measuring color in bread, *Cereal Chem.* 6, 60-68, 1929. M. C. Markley, Method of preserving bread for permanent grain judging standards, *Cereal Chem.* 11, 200, 1934. L. D. Whiting, Report of the 1935-36 committee on cake-baking tests and self-rising flours, *Cereal Chem.* 13, 736-743, 1936.

Slices cut from the cold biscuits were trimmed to the desired size and thoroughly air-dried between clean, white blotters, under enough weight to keep them flat without crushing. These dried "chips" were preserved by dipping for eight seconds in a solution of 30 c.c. U. S. P. flexible collodion in 75 c.c. ethyl alcohol and 195 c.c. ethyl ether and redrying between the blotters under the weight. The slices were attached to the glass by means of liquid adhesive and the backs covered with strips of adhesive tape which were coated with several applications of clear shellac. This gives a standard color chart capable of being handled without destruction or soiling.

### Experimental

Four carefully matched color charts were used in the collaborative work in 1937. A color chart and four samples of leavened flour containing shortening were sent to each laboratory. Directions were given for hand mixing the biscuits and for preparing the slices for color scoring.

The flours were baked and scored independently by two or more persons in each of the collaborating laboratories. The charts were then returned to the authors' laboratory where samples of the same flours were baked and scored against each color chart respectively and independently by four individuals. One color chart was returned too late to be included in the averages of all observations (Table I).

TABLE I  
AVERAGES OF CRUMB COLOR SCORING RESULTS ON 1937 SAMPLES AS  
REPORTED BY ALL COLLABORATORS

Chart No.	Flour A	Flour B	Flour C	Flour D
2	6.2	2.9	1.5	4.7
3	6.0	2.9	1.5	4.8
4	5.9	2.6	1.3	4.8

Since results in Table I may have been influenced too much by the skill developed in the authors' laboratory, the following averages are given to show the results obtained in the collaborating laboratories alone.

TABLE II  
AVERAGES OF CRUMB COLOR SCORING RESULTS ON 1937 SAMPLES AS  
REPORTED BY ALL COLLABORATORS EXCEPT THOSE IN AUTHORS'  
LABORATORY

Chart No.	Flour A	Flour B	Flour C	Flour D
2, in Lab. 2	6.7	3.0	1.7	4.7
3, in Lab. 3	6.1	3.2	1.9	5.2
4, in Lab. 4	6.0	2.8	1.4	5.2

The results in Table II reflect both the differences in judgment of the collaborators and any slight differences between the charts.

These four samples of flour previously used in 1937 were preserved in tightly closed cans and baked after a period of twelve months. They were scored against the original color charts, which had been kept in the dark. Results (Table III) showed a general dropping of color values from  $1\frac{1}{2}$  steps in the darker end of the board to 3 steps in the lighter end.

TABLE III  
AVERAGES OF RESULTS OBTAINED AFTER TWELVE MONTHS' STORAGE,  
USING FLOURS AND CHARTS PREPARED IN 1937  
(Compare with Table I)

Chart No.	Flour A	Flour B	Flour C	Flour D
2	7.5	5.0	4.5	6.0
3	7.2	5.5	5.0	6.0
4	7.5	5.5	4.3	6.5

In 1938 a single new color-standard board was used by all the collaborators. Special precaution was taken to keep this in a light-proof box when not in use. Two samples of flour, containing baking chemicals and shortening, were baked by collaborators and scored against this standard board. The results (Table IV) show gratifying agreement.

TABLE IV  
RESULTS OF COLLABORATIVE STUDIES, 1938, SAME STANDARD USED BY  
ALL COLLABORATORS

	Collaborators								
	A	B	C	D	E	F	G	H	I
Flour E	1	$1\frac{1}{2}$	$1\frac{1}{4}$	$1\frac{1}{2}$	1	1	$1\frac{1}{2}$	1	1
Flour F	2	2	2	$2\frac{1}{4}$	2	2	$2\frac{1}{2}$	2	$1\frac{1}{2}$
Average for Flour E	....			....	1.19				
Average for Flour F	..			.....	2.03				

In order to determine the effect of sunlight on the preserved slices two identical color charts were prepared and used in scoring two samples of flour. One of the two charts was immediately placed in a light-proof box, and the other exposed continuously to daylight and sunlight on a window sill. At short intervals the two test flours were baked and scored against the two color-standard charts. Within two days the chart exposed to the sunlight showed lighter and at the end of one week had dropped  $2\frac{1}{2}$  steps. At the end of one month readings had not changed further.

TABLE V  
 READINGS USING STANDARD CHARTS WITH ONE KEPT IN DARKNESS  
 AND ANOTHER EXPOSED TO SUNLIGHT

	Stored in darkness			Exposed to sunlight		
	2 days	1 week	1 month	2 days	1 week	1 month
Flour E	1	1	1	2	3½	3½
Flour F	2	2	2	3	5½	5½

### Conclusions

This method of scoring crumb color of biscuits is satisfactory for use within a short period of time if special attention is given to protecting the standard board from light. The method, however, is not satisfactory over an extended period of time.

### Acknowledgment

The authors wish to acknowledge the help given by the members of the committee and their associates who acted as collaborators.

## 1937-1938 REPORT SUB-COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

PEARL BROWN, *Chairman*

Perfection Biscuit Company, Fort Wayne, Indiana

(Read at the Annual Meeting, May 1938)

The 1937-38 sub-committee planned its work to carry out, in so far as possible, the recommendations of its predecessor (Cereal Chemistry 15: 35-48) as follows:

1. "That more work of the type herein reported be done to build up a greater volume of data to correlate more accurately the laboratory analyses of biscuit and cracker flours with their shop performance."

2. That "experimental work be undertaken to explain, and if possible correct, the apparent discrepancy between the baking test and the viscosity test for determining the relative strengths of biscuit and cracker flours."

### Procedure

The committee followed the procedure outlined by the previous committee. Committee members submitted analyses of flours with which they were familiar and which might be suitable for study. From

these lists four flours were selected. Each flour, so coded that its identity was hidden, was submitted twice to each committee member. The first time, determinations of moisture, ash, protein, and viscosity were made, and from these data the flours were classified as sponge or dough flours. The second time committee members were asked to run baking tests alone and to classify the flours from the results.

Crackers were made in the shop, also, according to the method used by Reiman (1938); that is, the two sponge flours were baked separately with the two dough flours, making four bakes. This series was tested three times at intervals of from three to four weeks. After each bake, a one-pound package of each type of cracker was sent to each committee member for scoring. On the first two bakes topping salt was omitted from the crackers. One member was asked to analyze the crackers for moisture, protein, and fat content, and another was asked to determine the shortometer values.

For the sake of continuity, the four flours used in this study are designated as 5, 6, 7, and 8, respectively. Table I shows the type of wheat from which each flour was milled.

TABLE I  
AVERAGE ANALYTICAL RESULTS ON THE FLOURS STUDIED

Sample no.	Used for	Wheat type	Ash at 15% H <sub>2</sub> O	Protein at 15% H <sub>2</sub> O	Viscosity		
					No acid	1 c.c.	7 c.c.
5	Sponge	Red	0.396	8.05	16.4	29.6	58.5
7	Sponge	Red	0.398	8.45	16.0	38.2	70.8
6	Dough	Red and white	0.378	7.86	16.6	33.3	56.0
8	Dough	Red and white	0.358	7.75	18.2	34.8	46.5

The methods for determining moisture, ash, and protein were the usual ones. The method for the viscosity test was that of Bayfield (1936), procedure 2.

The shortometer test was the same as was used last year, Reiman (1938), with the exception that only 52 readings were made on the first series of four crackers, 168 on the second, and 145 on the third series.

### Commercial Cracker Bakes

The procedure of the previous committee was followed as closely as possible. The differences were essentially those that might be expected to be encountered in different shops. Three series of bakes were made at intervals of from three to four weeks. Each series was composed of four five-barrel doughs. Each of the two sponge flours was baked with each of the two dough flours. Sixty-percent sponges were made, 50% of the total flour being incorporated in the sponge,



and 50% in the dough stage. The last cracker made was an exception. In it, 60% or all of the sponge flour was used in the sponge, and 40% or all of the dough flour in the dough.

The sponges, set at 70° F., were fermented 20 hours in a fermentation room maintained at 84° F. with the relative humidity between 50% and 60%. On the first bake, because of a condition in the shop, cracker No. 4 had 22 hours in the sponge stage. In the last bake, cracker No. 5, the sponge was set at 68° F.

Other conditions were maintained as nearly uniform as possible throughout the experiments. The doughs were mixed, fermented,

TABLE II  
CLASSIFICATIONS OF FLOURS MADE ON BASIS OF ANALYTICAL TESTS  
INCLUDING VISCOSITY

Collaborator	Flour 5	Flour 6	Flour 7	Flour 8
ARMUTH EVERT	Dough Dough or sponge	Dough Dough or sponge	Sponge Sponge	Dough or cookie Dough or cookie
HOLLINGSHEAD	Dough	Dough	Sponge	Strong sweet goods and cracker dough
KNUDSON	Dough or hard sweets	Dough	Sponge	Cookie-cracker dough
LOVING	Sponge dough or hard sweets	Dough—could be sponge	Sponge	Dough
OPPEN	Dough or sponge	Sponge or dough	Sponge	Dough or sweet goods
SKAER TRIEBOLD	Dough Dough or sponge	Dough Sponge or dough	Sponge Sponge	Dough Dough

TABLE III  
CLASSIFICATIONS OF FLOURS MADE ON BASIS OF BAKING TESTS

Collaborator	Flour 5	Flour 6	Flour 7	Flour 8
ARMUTH EVERT	Dough Dough and cookie	Dough Dough and cookie	Sponge Dough or cookie	Cookie Cracker sponge
HOLLINGSHEAD	Strong sweet goods and cracker dough	Very good cracker dough	Weak sponge or strong dough	Cookie
KNUDSON	Dough and hard sweet	Dough	Sponge	Cookie
LOVING	Dough—may be border line	Dough, hard sweet	Dough or hard sweet	Sponge
OPPEN	Dough or hard sweet	Dough	Sponge or dough	Sponge
SKAER	Dough	Dough	Sponge	Dough
TRIEBOLD	Dough or cookie	Dough	Sponge	cookie Sponge or dough

TABLE IV  
CHEMICAL AND PHYSICAL ANALYSES OF CRACKERS <sup>1</sup>

Bake no.	Cracker no.	Moisture as rec'd	Protein $N \times 6.25$	Fat	Shortometer value <sup>2</sup>	Av. pH	Thickness of 10	Count per pound
		%	%	%			In.	
I	1	3.7	9.0	10.4	70.2	8.0	$2\frac{7}{8}$	140
	2	3.7	9.1	9.0	72.5	8.1	$2\frac{7}{8}$	140
	3	2.8	8.9	11.1	76.3	8.2	$2\frac{7}{8}$	140
	4	3.1	9.0	11.7	56.4	8.1	$2\frac{7}{8}$	140
II	1	2.7	9.2	8.0	67.1	8.2	$2\frac{15}{16}$	140
	2	4.3	9.1	9.4	71.3	7.9	$2\frac{15}{16}$	140
	3	3.9	9.2	7.3	62.0	8.3	3	144
	4	3.9	9.0	8.0	65.1	8.2	$2\frac{15}{16}$	140
III	1	4.9	8.75	10.7	80.0	8.2	3	138
	2	4.4	8.90	10.4	72.0	8.3	$2\frac{15}{16}$	140
	3	4.2	8.75	7.8	74.0	8.3	$3\frac{1}{16}$	142
	4	4.8	8.50	9.9	79.0	8.4	3	142
	5	4.3	8.67	9.5	70.0	8.3	$2\frac{7}{8}$	139

<sup>1</sup> Chemical analyses by T. E. Hollingshead.

<sup>2</sup> Shortometer values by H. J. Loving.

TABLE V  
COMPOSITE OF SCORES AND SHORTOMETER READINGS

	Collaborator's ranking				Shortometer reading			
Cracker no.	1	2	3	4	1	2	3	4
Total	5	9	8	8	9	8	7	6
Rank	1	3	2	2	4	3	2	1

proofed, and baked under conditions similar to those reported by last year's committee.

Crackers were scored by each collaborator and the results evaluated as outlined in Cereal Chemistry 15: 37.

### Discussion of Results

The analytical data and the classifications made therefrom checked fairly well. As has been observed in the past, differences in moisture are due, no doubt, to varying conditions encountered in transit and in the laboratories before analyses are made.

Ash results indicated that flour No. 8 was lowest and flour No. 5 highest, the range in the corrected values between the two extremes being 0.04%. The range in protein was 0.7%. The flour lowest in protein was also lowest in ash, while the highest protein flour was also highest in ash.

Possibly the greatest variations occur in the reported viscosity results. Several of the committee suggested that discrepancies might

be attributed to a lack of standardization of the viscosimeters used. However, the classification of the flours from the analytical data showed splendid agreement among collaborators.

The results of the baking tests did not afford so favorable a comparison. The percentage of absorption, the loaf volume, and the characterization of all the features of the test loaves exhibited variations. It is rather surprising that, though the variations are there, the resulting classifications are in good agreement except in the case of flour No. 8. That flour was rated everywhere from a cookie flour to a sponge flour. No definite reason for such a wide variation in the classification of flour No. 8 has been advanced.

The two immediately preceding committees reported that wherever there was a difference in the rating of the flours by the two methods of testing, *i.e.*, viscosity and the baking test, the rating by the baking test was "a degree higher than by the viscosity test." The results on flours studied this year did not confirm that observation.

H. O. Triebold, in his comments on the characterizations made for flour No. 8 by the two sets of tests, has made some interesting observations which are as follows:

"Flour No. 8 gave the lowest values for ash, protein, and viscosity, after the addition of 7 c.c. of lactic acid and, on this basis, was rated as a dough or cookie flour.

"On the basis of the baking test, flour No. 8 was rated anywhere from a cookie to a cracker sponge flour. It is interesting to note that if the viscosity values on this flour be taken after the addition of 1 c.c. of lactic acid, this value is nearly that of the strongest flour, No. 7.

"A somewhat similar case may be noted in last year's report, *Cereal Chemistry* 15: 35-48. There, flour No. 2 had a much higher viscosity with 1 c.c. of lactic acid added than flour No. 4, yet with 7 c.c. added both had the same viscosity. However, flour No. 2 was a stronger flour than flour No. 4. There is apparently a much closer relationship between viscosity with 1 c.c. of lactic acid added and the baking test than there is after the addition of 7 c.c. of lactic acid."

These observations indicate that a further study of this correlation might be of value. Comments by other committee members indicated preferences for certain tests, as follows:

One said, "Personally, I would rather depend upon my own classifications from the analytical tests because, being in a mill laboratory and not in a cracker plant, I have been supplying flour for specific purposes by analyses." Another writes, "I may be a bit prejudiced, however, in feeling that the chemical tests are more valuable than the baking test." A third comments, "Frankly, I do not have much confidence in the baking test alone for judging cracker flours. My

vote goes to the viscosity test as the final instrument on which to base any classification for flour for crackers or sweet goods." These comments indicate a need for further study of the possible existence of closer correlation.

The results of the cracker scores were not too encouraging. Although the performance of the flours in the shop was satisfactory, and the crackers produced met the shop requirements, a definite lack of uniformity between bakes was indicated by the collaborator's scores and by the shortometer.

Reasons for this condition are still a subject for investigation. Even when the rankings of the three bakes are composited as were those of last year's committee, there is agreement only in the ranking of crackers numbered 2 and 3. Cracker No. 3 was ranked second and cracker No. 2 was ranked third. Cracker No. 1 was rated first by the collaborators' score and fourth by the shortometer. Cracker No. 4 was ranked second by the collaborators' scores and first by the shortometer test.

With the viscosity results used as the means of classification for the flours from which the crackers were baked, the flours used in the various cracker mixes are characterized as follows:

*Cracker 1* was made from the stronger sponge flour and the weaker dough flour. *Cracker 2* was made from the stronger sponge flour and the stronger dough flour. *Cracker 3* was made from the weaker sponge flour and the weaker dough flour. *Cracker 4* was made from the weaker sponge flour and the stronger dough flour.

With the baking test used as the means of classification:

*Cracker 1* was made from the stronger sponge flour and a dough flour that showed variable strength. *Cracker 2* was made from the stronger sponge and the stronger dough flour. *Cracker 3* was made from a flour in the sponge rated almost unanimously as a dough flour, and a dough flour that five of the committee rated as a sponge flour. *Cracker 4* was baked from the same flour in the sponge as cracker No. 3 and the strongest dough flour.

Because of the varied characterization of flour No. 8, the fifth dough in the last series was run with that flour in the sponge and the strongest dough flour in the dough stage. The sponge was set 2° cooler than the others. While the crackers resulting from this last mix were fairly satisfactory, commercially, they were not so good as some of the others. Compared with other crackers baked in the last group the shortometer ranked cracker No. 5 first, but the collaborators ranked it fifth.

With the proper adjustment in fermentation, flour No. 8 could be used satisfactorily as a sponge flour but it would offer fewer problems

if used as a dough flour. This tends to corroborate the opinion of Reiman (1938) quoted below:

"On the basis of either the baking or viscosity tests as the measure of flour strength, the quality of the crackers seems to be more dependent upon the type of sponge flour than upon the type of dough flour used in its formula."

### Conclusion and Recommendations

The work of the committee this year has been essentially a repetition of the work of the previous committee. The scope of the work is still too limited and the results not sufficiently positive to warrant definite conclusions.

However the committee does consider that added evidence is offered in support of the value of the tests used in evaluating flours for biscuit and cracker work, and that the tests merit further study.

The committee recommends:

That further study be given to the possible correlation between the classifications of flour made from the baking test, on the one hand, and the viscosity test involving the addition of 1 c.c. of lactic acid as suggested by Triebold, on the other.

That further work be done to correlate more closely the laboratory evaluation of cracker flours with their shop performance.

That some study be directed to types of cookie flour.

### Acknowledgments

Appreciation of the work of the committee composed of Messrs. Armuth, Evert, Hollingshead, Knudson, Loving, Oppen, Skaer, and Triebold is gratefully acknowledged. The committee acknowledges its indebtedness to the Kroger Food Foundation for information regarding flours and sources of supply. The writer is personally indebted to Messrs. Garnatz, Putnam, Reiman, and Bayfield for their many helpful suggestions in planning the work. The crackers were baked by Perfection Biscuit Company under the supervision of Henry Hoffman and Herman Rang.

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## REPORT OF THE 1937-38 COMMITTEE ON METHODS OF TESTING SOFT WHEAT <sup>1</sup>

H. W. PUTNAM, *Chairman*

Igleheart Brothers, Incorporated, Evansville, Indiana

(Read at the Annual Meeting, May 1938)

During 1937-38, cereal chemists and their assistants in twenty-nine laboratories worked on projects of the Committee on Methods of Testing Soft Wheat. This committee was organized by the Executive Committee of the Association in May, 1937.

The sub-committee on Methods of Testing Cake Flour (J. W. Montzheimer, chairman) made a carefully planned collaborative study of the effects of five variations in the tentative A. A. C. C. cake-flour test. The results, printed in this issue of Cereal Chemistry, indicate that three flours can be rated in essentially the same order by each scheme of handling. Individual results, however, show the need for further study to clarify the scoring instructions to minimize the effects of the personal factor.

Since considerable dissatisfaction with the present procedure exists, and since it is not in common use, it is expected the next committee will investigate additional formulas and methods of handling.

Specifications for both loaf and layer pans were selected tentatively.

Mark A. Barmore co-operated with the sub-committee by determining in the altitude chamber at Fort Collins, Colo., the adjustments necessary for compensating the tentative formula for different air pressures from sea level to 10,000 feet.

The sub-committee on Testing Biscuit and Cracker Flours (Pearl Brown, chairman) continued the study of the usefulness of the viscosity and baking tests respectively in predicting the purpose for which a flour should be used. The practical significance of these conclusions was tested by commercial cracker bakings on the four flours used. It is expected that the succeeding committee will repeat this work on flours of known history in an attempt to eliminate variables which have crept into the work thus far, and to discover such correlations as may exist between the baking method and viscosity technique.

The sub-committee on Testing Self-Rising (and Phosphated) Flours (O. E. Gookins, chairman) undertook several individual projects for the purpose of improving the method for baking self-rising flours.

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<sup>1</sup> General report.

These included a simplification in the method of mixing, a study of oven variables, the determination of correct water absorption, a further definition and evaluation of various items on the score card, and a further study of preserved crumb-color standards. It remains for future committees to study the baking method and scoring system with flours as the variables and to determine as far as possible what steps must be taken to eliminate further the effects of personal opinion and skill in technique.

The study of pie flours should be included in the work of this committee. However, interest was lacking among those approached this past year.

While the work has been confined to those persons acknowledged in the committee reports, an invitation is extended to other interested persons who wish to work with the committee.

## WHAT THE A. A. C. C. BAKING TEST MEANS TO THE BAKER <sup>1</sup>

LAURA K. TRACK

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Collaborative work on standardization of the experimental baking test was so productive in the case of hard-wheat flours that the American Association of Cereal Chemists in convention at Omaha in June, 1927, proposed a committee for the purpose of study on the question of establishing a baking test for soft-wheat flours.

Preliminary questionnaires to committee members and others deemed particularly interested in research along this line brought out the fact that while many made chemical determinations on soft-wheat flours, they felt such tests were not adequate, some being in actual doubt as to just what these determinations reflected relative to the baking qualities of a flour. Most of those answering the questionnaires thought some sort of baking test necessary in evaluating soft-wheat flours. Many had adopted the bread-baking test because they were more familiar with it and because the bread-baking test was more standardized, but no one felt that satisfactory interpretation for cake work could be made from the bread test. For the most part no scoring method was applied.

Some used a cake-baking test, the greater number having selected a white-cake formula containing shortening, others a yellow cake,

<sup>1</sup> Report of 1937-38 sub-committee on Testing Soft-Wheat Flours.

while several used an angel-food formula. Formulas and procedures varied as well as methods of scoring. None of those using the cake-baking test had confidence in its reflection of qualities in flour for purposes other than cake.

Those using the biscuit-baking test were principally interested in biscuit flours and self-rising flours. Formulas were within a short range of variation, scoring was on more definite lines, the whole baking test seeming to be much more standardized than the cake-baking test.

It became obvious that a baking test to be used in conjunction with chemical determinations was necessary. And as the majority of opinions favored a cake-baking test the committee formulated a tentative test to determine whether or not such a test would prove useful in reflecting other factors than characteristics of flour.

From this time forward the committee on Methods of Testing Cake and Biscuit Flours (*Cereal Chemistry* 5: 301-309, 1928) has conducted collaborative tests, using suggested formulas and methods, and modifying formulas and methods in an effort to secure a test employing standardized materials as far as is feasible and having the least liability to errors through variability in time, manner of introduction of ingredients, panning, baking, etc.

The preliminary tests indicated a need for study on mixing, timing, method of scoring, and size and shape of pans. Collaborators working through subcommittees on the individual problems obtained results which led to the adoption in 1931 of the score card now used in connection with the A. A. C. C. baking test for soft-wheat flours. See score card and explanation of terms, *Cereal Chemistry* 8: 253, 1931.

Viscosity, specific gravity, and other phases of mixing having been studied from several angles by the several collaborators, the white-cake formula containing shortening and mixed by the single-phase method was adopted tentatively by the organization in convention in May, 1932 (*Cereal Chemistry* 9: 408, 1932).

Tolerance tests as to fat, sugar, and liquid were then added, the purpose being to show the strength of the flour in its ability to carry additional quantities of any of these or combinations of these in graded steps above the amount included in the original formula which served principally as an indicator of general baking characteristics of the flour being tested as to volume, texture, grain, color, etc. These latter tolerance tests pointed to the possibility of placing in certain ranges flours adapted to heavier types of cake and lighter goods according to tolerance to sugar, tolerance to fat, tolerance to liquid, etc., and made it possible for the commercial baker to determine in his laboratory the definite baking value of a flour before trying it out on a production basis.



In order to bring the results of the A. A. C. C. cake-baking test for soft-wheat flours more closely into correlation with results of commercial baking, frozen egg white was substituted for the egg albumin initially used in the tentative formula. Tests on the value of frozen eggs and reconstituted egg albumin carried on by collaborators showed conclusively that the frozen eggs more nearly paralleled fresh eggs, and that cakes made with frozen eggs were more truly representative cakes than sample bakings made with egg albumin and, therefore, more readily translated into terms of general production.

For general routine purposes this test may be used by the baker to check on new lots of ingredients. Very often flour will vary from lot to lot. Sometimes, while this is not enough to show up in the finished product, there are times when a simple preliminary test would check this variable and make it possible to adjust procedures accordingly. Sugar granulation very often affects cake as to crust characteristics, volume, texture, and grain. Again the laboratory test is an indicator as to what would happen in production. Shortening, too, might well be tested out first in this way. While a creaming test to a definite specific gravity is an excellent procedure to follow in general work after the creaming value and baking value of a particular lot of shortening have been determined, it is advisable to make a baking test as well since not all shortenings of the same creaming value register the same baking qualities. Eggs and milk also come under the heading of new-lot routine tests for the careful production man.

In using the A. A. C. C. baking test for soft wheat flours the Hobart C-10 mixing machine with adapter and three-quart bowls is recommended. Duplicate machines are an advantage. A comparative test is made on two ingredients, or methods, at one and the same time. Care must be exercised that the machines are synchronized as to speed and distance of paddle from bowl and these checked periodically in order to secure even volume results. Creaming shortening and checking volume or specific gravity against time are a good check on the proper set of duplicate machines. An occasional straight check baking for volume results is excellent.

Temperature becomes a very important part of such tests. Temperature control especially is more necessary in a series than in individual or single bakings. Such control may be accomplished by means of simple water baths, tempering of large batches near ovens, refrigeration, etc. It is advisable to keep records of temperatures of room, ingredients, batters, and oven, as these sometimes have important bearings upon volume, grain, texture, etc.

In using duplicate mixing equipment, the baking test becomes doubly interesting and revealing as cake from each of two mixes is baked at one and the same time. There is no chance for a variable oven temperature to bring about an effect in grain which, if the two were baked separately, might well be laid to the fault of the flour. Besides this there is the advantage of the composite cake which is unsurpassed for showing slight degrees in color differences and grain and texture. The composite cake is simply one cake made up of two batters—one-half of the weight of the total cake made up of one batter placed at one end of the pan and the remaining scaling weight made up of the second batter in the opposite end of the pan. In cakes of moderate consistency this is done easily without the aid of a temporary middle division to be taken from the batter before baking, as the two batters gradually meet in the middle of the pan and form one undivided cake. With thinner batters a temporary partition is advisable. When baked, this cake will show very clearly any differences in crust between the two mixes and, upon being cut lengthwise, will show any deviation in color, grain, or texture.

Score sheets in conjunction with simply made prints of cake slices make valuable records. For general purposes it is not always advisable to score numerically since these, having so much of the personal element entering into them, are not easily translated by others, especially upon reviewing records of work previously done. Short descriptive terms on grain, texture, color, etc., in addition to volume and weight figures, are more illuminating, especially when viewed in conjunction with the prints.

These prints may be made easily by means of stencil ink such as is ordinarily used in shipping departments, applied lightly and evenly with a paint brush to the cake slices, which in stamp-like fashion are lightly pressed, inked face down, upon paper and an impression thus obtained. Duplicate and triplicate prints may be made in this way. A slice from the middle of the cake, one half-way between middle and end, and the end slice when printed give a good cross section of the cake. When such prints are made on one sheet of paper from two cakes, together with a lengthwise slice from the composite cake of a comparative test, one has a record that, so far as volume, symmetry, grain, and texture are reflected, is more revealing than numerical scores.

Such tests are valuable in detecting differences from lot to lot of general basic ingredients and in bringing out differences between grades and types of materials. Flour differences are reflected in the type of grain and texture obtained. The A. A. C. C. formula for testing soft-wheat flours will show differences even in the batter stage

between flours of varying protein contents and will register in the baked cake differences in volume, grain, and texture, which will indicate changes advisable in manufacture by varying liquid, shortening, or sugar. Together with the tolerance tests, which are carried out by increasing the percentages of sugar, shortening, and liquid to the point of breakdown, this test places a flour for use in rich mixes such as pound or other heavy types, or light layers and cups, etc. Flours which have low sugar tolerances would indicate a cookie type of goods. Differences in granulation within the same type of flour will be reflected in the tolerance test, coarser granulations as a rule having less tolerance to shortening and sugar. Likewise the extent of aging of the flour influences the tolerance to liquid, shortening, and sugar and may be gauged by the baking test in comparison with a flour of known value, the greater extent of aging within certain limits enhancing the volume and tolerances of a flour.

As an example, suppose we were considering the purchase of flours that appeared to us to have merit along with a saving in cost but with which we had had no baking experience. A baking test by the A. A. C. C. method on samples furnished by the miller would show the qualities of these flours in comparison with a flour of known value and would give us actual knowledge of the flours upon which to base our decision.

The flours in question when baked and scored according to the A. A. C. C. method might line up as follows:

Ideal Score		Known Flour or Now in Use	Flours under Consideration	
			A	B
<i>A. External</i>				
1. Symmetry	10	9.5	10.0	9.0
2. Volume	15	14.5 (771 c.c.) <sup>1</sup>	15.5 (792 c.c.)	19.5 (870 c.c.)
3. Crust	5	4.0 sugary	4.5 deeper colored, sugary	4.5
<i>B. Internal</i>				
1. Texture	30	20	25	28
2. Grain	25	22	20	18
3. Color	15	14	14	14.5
Total	100	84	89	93.5

When tested for shortening tolerance by increasing the amount of shortening in the original formula by 50% they would perhaps reflect the following:

Ideal Score		Known Flour or Now in Use	Flours under Consideration	
			A	B
<i>A. External</i>				
1. Symmetry	10	9.0	9.0	9.75
2. Volume	15	13.5 (749 c.c.) <sup>1</sup>	16.0 (804 c.c.)	17.5 (828 c.c.)
3. Crust	5	3.0 macarooned	4.75 crust not as sugary	4.5 very slightly crinkled
<i>B. Internal</i>				
1. Texture	30	26	28	30
2. Grain	25	21	22	21
3. Color	15	14.5	14.75	15
Total	100	87.0	94.5	97.75

Under the sugar-tolerance (20% increase) test these flours might rate as follows:

Ideal Score		Known Flour or Now in Use	Flours under Consideration	
			A	B
<i>A. External</i>				
1. Symmetry	10	9.5	9.5	10.0
2. Volume	15	14.5 (776 c.c.) <sup>1</sup>	16.0 (797 c.c.)	17.5 (827 c.c.)
3. Crust	5	3.5 more sugary	4.5 slightly brownier, tenderer	4.75 tenderer, not sugary
<i>B. Internal</i>				
1. Texture	30	23.0 trifle furry	25	29 velvety
2. Grain	25	22	21	20
3. Color	15	14	14	14
Total	100	86.5	89	95.25

<sup>1</sup> Volume figure of 780 c.c. taken as representing 100% volume count of 15. For each 20-c c. variation, one point is added or deducted according to whether the volume is larger or smaller.

By straight volume test flour *B* is a stronger flour than *A*, which though more closely approximating the *known flour* shows better general characteristics except for color.

Under the shortening-tolerance test of 50% increase, the *known flour* falls off in volume almost 3%, while *A* becomes greater by approximately 1%, and *B* falls off by nearly 5%.

Using the sugar-tolerance test of 20% increase, the volumes of the known flour and flour *A* remained normal, flour *A* showing greater tolerance by its crust, which is tenderer and slightly browner than the known flour, which produces a more sugary exterior. Flour *B* falls

off in volume under sugar stress as it does under the increased shortening (approximately 4.5%) but shows a tenderer crust, not sugary.

Texture is improved for all three flours by the increase in shortening, the known flour showing greater improvement than flour *A* and flour *B*. Grain is improved to the same extent in flours *A* and *B*, while it falls off very slightly in the case of the known flour.

Flour *B* shows slightly better color than the others in the initial test and the shortening tolerance test; however, under the sugar-tolerance test, while not inferior to the others it is not as good in color as without the increased sugar.

Flour *A* shows a very slight increase in volume when shortening is increased. Both *A* and *B* show marked improvement in texture with the increased amount of shortening, *B* having definitely more velvety texture. Color improvement is also evident in both cases, the development of color and texture being more marked than with increased sugar content.

Flour *A* gives crusts of glossy, sugary character while *B* gives crusts that are deeper in color without the hard gloss of *A* but inclined to crepiness with increased shortening, but tenderer throughout.

Flour *B* shows qualities for velvety texture but the grain is a trifle uneven. The texture of *A* is on the feathery to furry side.

Flour *A* would most probably be best in lighter-cake mixtures, layers, cups, sponge, and angel cakes. Flour *B* would show to better advantage in heavier-type cakes, such as pound and fruit.

The effect of sugar granulations may also be reflected by means of such a baking test, and production changes made accordingly.

Comparative values of different shortenings may also be gauged by this baking test, some tending to produce larger volumes, some greasiness, others waxiness, still others oiliness, any of which would be more or less indicative of advantages in special classes of baked products.

The A. A. C. C. baking test for soft-wheat flours promises to become a dependable gauge in the laboratory of the alert baker who is interested sufficiently to develop the power of observation and translate into production lines the guide posts the test reveals.

## ALTITUDE VS. BAKING POWDER USING TENTATIVE A.A.C.C. CAKE FORMULA

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(Read at the Annual Meeting, May 1938)

The purpose of the experiments herein described is to find out the amounts of baking powder necessary to produce the same grain, volume, contour, and general characteristics in cakes at different altitudes.

The formula and procedure used are as published on pages 75 to 80 of Cereal Laboratory Methods, third edition, except that fresh egg albumen and a small home-type mixer were used. (Results were, however, the same as those obtained with the Hobart mixer.) All cakes were baked 45 minutes at 183° C. (362° F.) except those at the 10,000 foot level, which were baked 55 minutes in order to eliminate the soggy streak appearing beneath the crust. All tests were run on one brand of commercial cake flour, but the final results were found to check very satisfactorily when another type of commercial cake flour was used.

A series of cakes was baked at each altitude given in Table I. The ones chosen as optimum gave the maximum volume and still maintained very satisfactory texture and grain characteristics, which were similar to those obtained with the A. A. C. C. tentative formula at sea level. An example is shown in Figure 1. The lines appearing are produced by a screen placed over the samples to serve as a reference scale, each square having an area of 4 square centimeters.

The amount of cream of tartar which produced the most satisfactory cake is given in Table I and can be expressed by the equation  $y = -0.034^2 - 0.25A + 6$  where  $y$  equals the cream of tartar in grams and  $A$  the altitude in thousands of feet. The specific gravity of the

TABLE I

Altitude	Cream of tartar	Soda	Sp. gr. batter	Weight of cake	Volume of cake	Sp. gr. cake
Sea level	6.0	3.00	0.92	272.5	780	0.349
5,000 ft.	4.0	2.00	0.91	267.3	820	0.326
7,200 ft.	2.5	1.25	0.91	262.0	790	0.331
10,000 ft.	0.5	0.25	0.92	260.5	715	0.364

batter shown is that obtained just previous to the addition of the cream of tartar, and for the entire series at all altitudes it ranged from 0.89 to 0.94 g. per c.c.

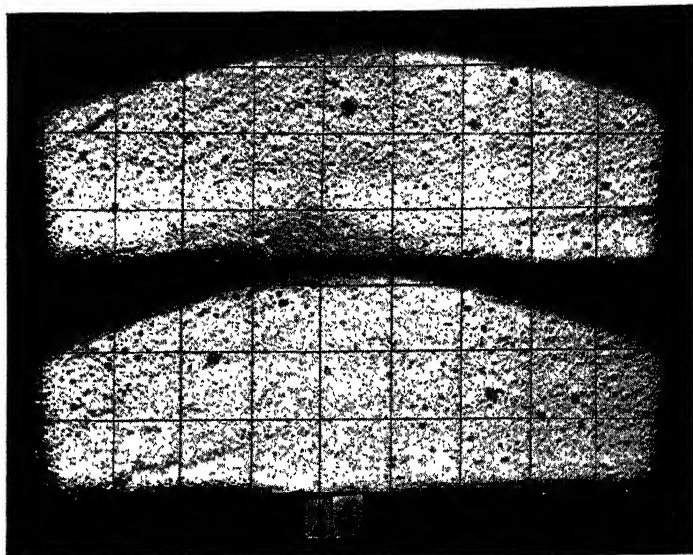


Fig. 1. Baked at sea level with the A. A. C. C. formula plus 30 g. of sugar.

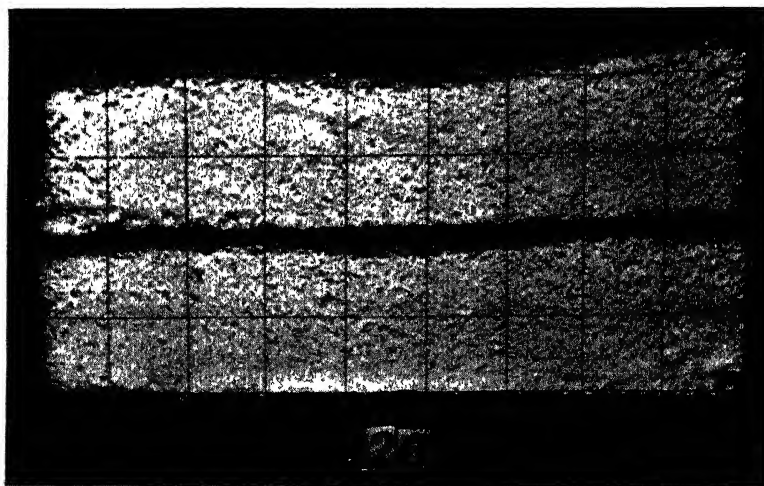


Fig. 2. Same as Figure 1 but baked at an altitude of 10,000 feet.

In order to show that the sugar tolerance is entirely different for the same flour at different altitudes, cakes were baked at sea level and at

10,000 feet using the A. A. C. C. tentative formula with an increase of 30 grams of sugar. Those baked at sea level (Fig. 1) gave excellent results, but the same cake baked at 10,000 feet fell flat (Fig. 2), although the correct amount of baking powder according to Table I was used. It would appear that at higher elevations it will be necessary to work out a chart for the sugar tolerance as well as the difference in the amount of baking powder required.



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## QUANTITATIVE METHODS FOR EVALUATING THE QUALITY OF MACARONI PRODUCTS <sup>1</sup>

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(Read at the Annual Meeting, May 1938)

The term "quality" as applied to macaroni products does not possess absolute significance and can only be defined on the basis of the factors contributing to consumer preference. The characteristics of a good macaroni have been defined by LeClerc (1933) as hardness, brittleness, translucency, elasticity, and a rich amber color. The fracture should be glassy and long pieces should be sufficiently pliable to allow of considerable bending before breakage. In addition, the behaviour upon cooking is most important; LeClerc states that when boiled for ten minutes, "a good macaroni will swell to at least twice its original size, will retain its tubular shape and its firmness, will not become pasty, and will have an agreeable odor." The factors associated with quality may thus be classified into three groups:

- (1) Color and related factors such as vitreousness and translucency.
- (2) Mechanical strength.
- (3) Cooking characteristics, including water absorption, swelling, disintegration, and tenderness.

In a general way, the quality of macaroni may be assessed by means of visual examination and a simple cooking test, but such methods lack quantitative significance and are valueless when applied to the estimation of comparatively small differences. In the present paper, the development of quantitative methods is outlined and the interpretation of the values obtained is discussed.

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### Color

In countries where the addition of artificial coloring matter is prohibited, color appears to be the principal basis of consumer preference and at the present time it is the most widely used quality index. The factors associated with a desirable macaroni color are quite complex, involving not only the pigment content but also translucency and vitreousness which, in turn, are apparently dependent upon the quantity and quality of the proteins and the degree of hydration of the starch. Wide variations in color can also be introduced by differences in processing technique as shown by Binnington and Geddes (1936). Accordingly, this quality factor cannot be adequately evaluated by any single analytical test, such as carotene or protein, and a direct determination is essential.

Measurement of macaroni color was first carried out in this laboratory by means of a spectrophotometer, employing discs cut from a flat strip produced by means of a special die. Material of this type is almost essential if a spectrophotometer is to be employed, and the method is therefore limited in its application. In addition, as indicated in an earlier study (Binnington and Geddes, 1936), the method is slow, the calculations involved are very laborious, and the results obtained are not readily interpreted in terms of visual color. In view of these limitations, the method was ultimately abandoned and attention directed to the use of a matching-type procedure, using the original-model Wallace and Tiernan colorimeter and discs as described by Baker, Parker, and Freese (1933) with a daylight lamp as illuminant. Later, a Bausch and Lomb Type H.S.B. Color Analyzer was secured, and the majority of color analyses conducted have been made with this instrument. Munsell discs have been used very largely, the following selection having been found to cover the majority of samples encountered:

Durum Semolina—Y — Y.R. 8/6, Y 8/12, N 9.4 and N 8.

Durum Macaroni and Spaghetti—Y.R. 6/12, Y 8/12, N 7 and N 4.

In expressing the results obtained with these discs, values for "hue," "brilliance," and "saturation" are computed according to the formula outlined by Nickerson (1929), and from these data an arbitrary single figure estimate of color has been derived as follows:

$$\text{Single-figure color score} = \frac{\text{hue}}{(\text{brilliance/saturation})}.$$

With varietal material, this arbitrary single-figure estimate of color has been found to yield results in excellent agreement with a careful visual classification; furthermore, it has been found possible to compare, directly, results obtained over a period of years.

In certain cases, particularly where graphical presentation is desired, Wallace and Tiernan color discs may be employed to decided advantage (Binnington and Geddes, 1937). However, such graphical expressions do not integrate the color constituents and it is necessary to accomplish such an integration in order to secure quantitative figures which will relate to visual appearance. For example, the arrangement of a series of samples on the basis of percentage of yellow alone will not correspond to a visual classification if the ratio of yellow to red varies, because the red component imparts a brownish characteristic to the color; similarly, the white and black components influence the visual appearance. Accordingly, an effort has been made to integrate the N. A. disc values into a single-figure color score. The initial step is to express the percentage readings of the four discs in terms of hue, saturation, and brilliance. This computation can be made from a knowledge of the Munsell equivalents of the N. A. discs but the calculation is exceedingly laborious and is otherwise unsatisfactory because of the wide separation in Munsell hue of the red and yellow N. A. discs. For these reasons it is deemed preferable to compute arbitrary indices of hue, saturation, and brilliance as follows:

$$\begin{aligned}\text{Hue} &= \% \text{ yellow} / \% \text{ red.} \\ \text{Saturation} &= (\% \text{ yellow} + \% \text{ red}) / \% \text{ black.} \\ \text{Brilliance} &= \% \text{ white} + \% \text{ yellow.}\end{aligned}$$

From the above values a single-figure estimate of color is computed by the arbitrary formula:

$$\text{Single-figure color score} = 2(\text{hue} \times 5 + \text{saturation} \times 2 + \text{brilliance}/4).$$

In this formula an attempt has been made to weight the various components according to their relative significance as regards visual appearance; it differs from that employed in computing a single-figure score from the Munsell disc data because the indices designated as "hue," "saturation," and "brilliance" are purely arbitrary and their magnitudes bear no relation to the corresponding values for the Munsell discs.

As the two systems of computing a single-figure color score are based on different data and employ different weightings, no direct comparison can be made between the scores which, in addition, are of quite different magnitudes. The Munsell disc method gives values on experimentally processed durum macaroni ranging from 12 to 25 units and has been found especially suited to studies involving varietal material; the N. A. disc procedure yields scores ranging from 50 to 100 units and appears better adapted to studies on samples where differences in hue are slight and saturation and brilliance are the principal factors responsible for color variations.

The actual color measurements are made upon a layer of the material of sufficient thickness to eliminate any "background" effect and with the Bausch and Lomb instrument the sample is not rotated.

### Mechanical Strength

A high degree of mechanical strength is desirable in macaroni products in order to minimize breakage. As indices of mechanical strength, measurements of tensile strength, crushing strength, and transverse and torsional breaking strength might be carried out but, for macaroni products, a test of the transverse breaking strength appears the most suitable. Tensile-strength tests are not feasible because of the difficulty of clamping without breaking; crushing tests would require sensitive methods of measuring the small loads required for such fragile material, while torsional tests are only applicable to spaghetti or other products of similar diameter.

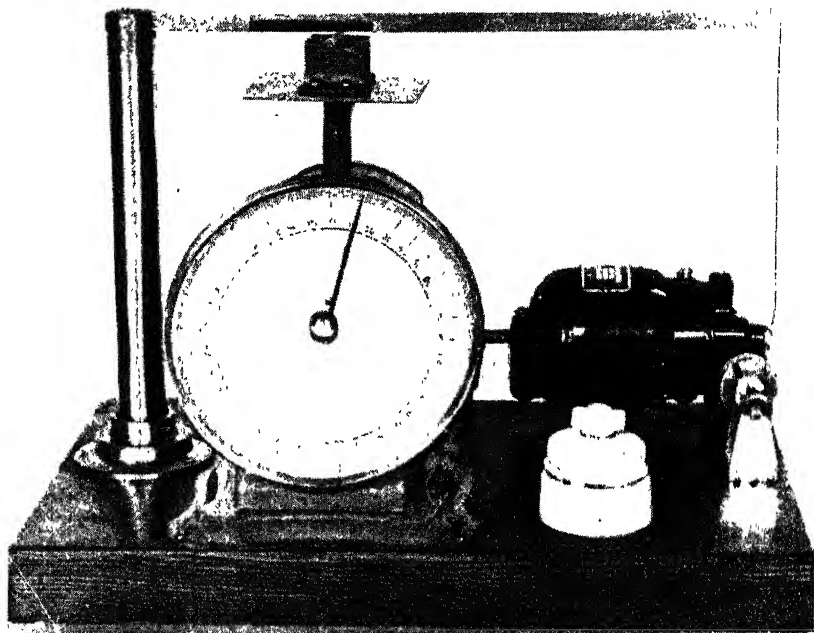


Fig. 1. Breaking-strength tester.

The breaking-strength apparatus employed is illustrated in Figure 1 and is modeled along the lines of the Bailey shortometer (1934). The sample is placed across two supports mounted on the pan of a 24-pound dial-indicating spring scale, the load being applied by means of a pivoted

bar driven through the medium of a cord and winch from a slow-speed (5 r.p.m.) motor; a non-return pointer is fitted to indicate the breaking point. The dial of the scale was replaced by one graduated in angular degrees and the results obtained have been recorded directly in these units.

The accuracy of the test was studied by means of 20 replicate tests conducted upon each of five replicate processings of macaroni. The results are summarized in Table I, and show that there is considerable

TABLE I  
BREAKING STRENGTH OF MACARONI FROM FIVE REPLICATE PROCESSINGS

Batch No.	Mean breaking strength (20 readings) arbitrary units	Variance analysis				
		Variance due to:	D.F.	Variance	F.	5% pt.
1	152.3	Differences between batches	4	6348.46	19.70	2.46
2	151.0	Differences within batches	95	322.23	—	—
3	193.4	Total	99	565.71	—	—
4	174.8	Standard error of single observations	= 23.78.			
5	175.4	Standard error of means of 20 readings	= 5.32.			

variation between replicate tests made on the same batch and also that there is a significant difference between batches. Taking into consideration both the variation within and between batches, the above study gives a standard error for the means of 20 determinations of 5.32 units in breaking strength, which implies that the differences between the means of 20 tests for any two different macaronis must equal or exceed 16 units in order to be significant.

In view of the wide variation in the breaking strength of different portions of the same strand, an extensive series of tests was made in which breaking strength was compared with minimum and maximum wall thickness and diameter. A statistical analysis of the data, however, showed that differences in these dimensions were not the primary causes of variation in breaking strength. This result is rather surprising and suggests that the variations found are connected with the internal structure, as, in the case of such replicate tests, differences in composition are not involved. It was felt that some of the variations encountered might be due to some feature of the experimental press, such as irregularities in the dies or inadequate pressure. Measurement of the die

openings did not substantiate this hypothesis, and a limited number of tests made with high-grade commercial macaroni indicate that the variation is just as great with this class of material in spite of the fact that wall thickness and diameter are somewhat more uniform.

The relation between protein content and breaking strength was investigated with a series of macaroni samples processed from Canadian amber durum wheat of varying protein content and grade prepared by compositing a large number of envelope samples. The results presented in Table II indicate that breaking strength increases significantly with

TABLE II  
RELATION BETWEEN PROTEIN CONTENT OF WHEAT AND BREAKING  
STRENGTH OF MACARONI

Nos. 1 and 2 C.W. Amber Durum		Nos. 3 and 4 C.W. Amber Durum	
Protein content (13.5% M.B.)	Mean breaking strength	Protein content (13.5% M.B.)	Mean breaking strength
%	Units	%	Units
10.6	153.0	10.7	162.5
11.4	166.2	11.5	167.2
12.4	169.5	12.2	177.0
13.3	190.4	13.1	178.8
14.0	196.8	14.2	185.0
		15.1	190.5
Mean	175.2		174.1

increasing protein content and also that the rate of increase is greater in the instance of the higher grades. When the method was applied to a wide range of varietal material, however, no simple correlation could be discerned between protein content and breaking strength, and it would appear that other factors possibly associated with protein quality are also effective.

Comparative breaking-strength studies have also been conducted on macaroni processed from semolina, farina, and flours of varying extraction prepared from single samples of durum and hard red spring wheat. The results given in Table III show an increase in breaking strength from semolina or farina to flour. The increase in protein content is undoubtedly a contributing factor to the trends observed but the large increase in breaking strength of macaroni processed from 50% patent durum flour, as compared with the semolina which is only 0.4% lower in protein content, indicates that granulation is an important factor.

The breaking strengths observed with experimentally processed material range from 140 units to 200 units, whereas commercially

TABLE III  
BREAKING STRENGTH OF MACARONI PRODUCED FROM DURUM AND  
HARD RED SPRING WHEAT PRODUCTS

Basic material	Protein content (13.5% M.B.)	Macaroni breaking strength
	%	Units
Durum semolina	12.9	164
Durum flour 50% patent	13.3	182
Durum flour 60% patent	13.2	177
Durum flour 70% patent	13.8	180
Equal parts of semolina and 60% durum flour	—	166
Hard red spring farina	12.9	159
Hard red spring flour 50% patent	14.2	186
Hard red spring flour 60% patent	14.1	179
Hard red spring flour 70% patent	14.4	184

processed samples of similar diameter and wall thickness yield results in the order of 235 to 280 units. These differences suggest that factors involved in processing, such as pressure, etc., have an important influence upon the results and, unless the technique is carefully controlled, may easily mask differences due to composition.

### Cooking Characteristics

The cooking properties of macaroni are highly important and may be roughly defined as the ability to resist disintegration upon prolonged boiling with water, coupled with a satisfactory degree of tenderness in the finished product. Quantitative measurement of such characteristics is a very difficult problem, and tests of this kind have been usually confined to a visual estimate of turbidity in the cooking water, coupled with mastication of the macaroni, as an index of tenderness. Early attempts to evaluate these qualities quantitatively by active boiling were unsuccessful; the amount of disintegration found was exceedingly variable and no method was available for measuring tenderness.

Recently, the Italian investigator Borasio (1935) has published a valuable paper on the cooking characteristics of alimentary pastes, and details methods he has developed for their investigation. His technique has served as a basis for the procedure to be described, and, as the original paper is not readily available, this work is reviewed in some detail. Borasio lists the principal characteristics of interest from a cooking standpoint as:

- (1) Degree or amount of cooking required.
- (2) Resistance to disintegration.
- (3) Capacity for absorption of water.
- (4) Increase in the volume of the paste.



A paste of good quality possesses a notable cooking degree (*i.e.*, requires a relatively long time to cook), a high degree of resistance to disintegration, a large water absorption, and a considerable increase in volume. He points out that cooking tests made by active boiling are subject to considerable variation due to concentration and violent agitation, and outlines a test in which 250 g. of macaroni is cooked, without boiling, in 1 liter of 1% salt solution by means of an oil bath maintained at 105° C. The time in minutes required for complete cooking is taken as a measure of the cooking quality. Unfortunately, however, no indication is given as to the criteria employed to judge when cooking is complete.

In addition to ascertaining the cooking time, the water absorption is determined by draining for five minutes on a Buchner funnel and observing the increase in weight. Volume increase is measured by placing the cooked and drained sample in a specially designed volumeter and adding a known amount of water; the increase in volume is read from a graduated tube, a similar determination having been conducted with the uncooked material. Resistance to disintegration is estimated in an approximate manner by allowing the residual water from cooking to stand in a graduated cylinder and measuring the volume of deposited material. More accurately, the residual water is made up to definite volume and an aliquot evaporated to dryness on a steam bath in a tared beaker and dried to constant weight at 105° C., the presence of added salt being corrected for by a quantitative determination of chlorine. It is stated that with macaroni of good quality, the residue will not exceed 6%.

*Development of the test.*—In developing a test along the lines of the above procedure, it was felt that some method of measuring tenderness was essential, and as a preliminary, a tenderness tester was constructed, modeled along the lines of the instrument designed by Bonney, Clifford, and Lepper (1931) for canned fruits and vegetables. This device consists essentially of a plunger terminating in a circular metal disc which rests upon the sample to which a load is applied at constant rate by means of mercury until a predetermined reduction in sample thickness is obtained; the weight of mercury is taken as an index of the tenderness.

The major factors associated with the test were investigated with a modified form of this apparatus, and the following conclusions drawn:

- (1) It is necessary to take the mean of at least five replicate tests from a single cooking in order to secure a fair average.

- (2) A definite optimum time of cooking appears to exist beyond which excessive softening results.

- (3) Standing in water at room temperature for a moderate length

of time (30 minutes) after cooking does not affect the results appreciably.

(4) Small variations in macaroni temperature have no significant influence on the compression values.

(5) The presence of salt in the water employed for cooking results in increased tenderness for a similar cooking time. There is some indication, however, that the variability is increased.

The selection of a suitable thickness to which the sample should be compressed remained to be determined and, in an effort to investigate this, a test was made in which the load was applied in increments of 100 g., the reduction in thickness being measured after each addition. The results, calculated as percentage reduction in thickness and plotted against load, are shown in Figure 2.

The initial rapid drop represents the collapsing of the tube walls under the weight of the plunger and flask; compression then proceeds at a uniform rate over a considerable load-range and then increases rather rapidly. Obviously, the latter corresponds to a definite "yield" or "break" point, at which the sample gives way completely. A very important point disclosed by these studies was the effect of rate of

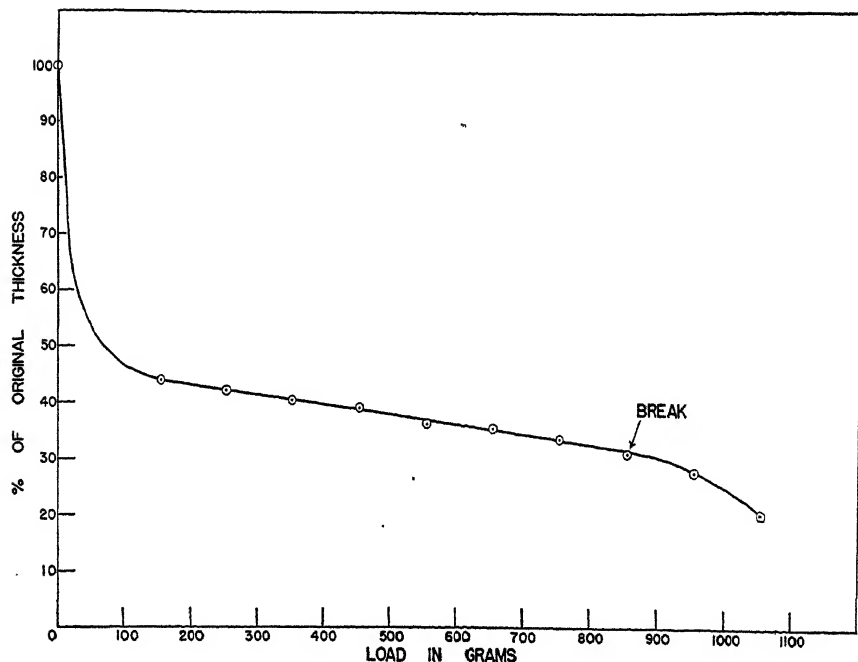


Fig. 2. Graph showing reduction in thickness of cooked macaroni with increasing load.

application of load, which must be quite uniform if comparable results are to be secured. This precluded routine application of the test in the manner described above and suggested the desirability of incorporating a recording device.

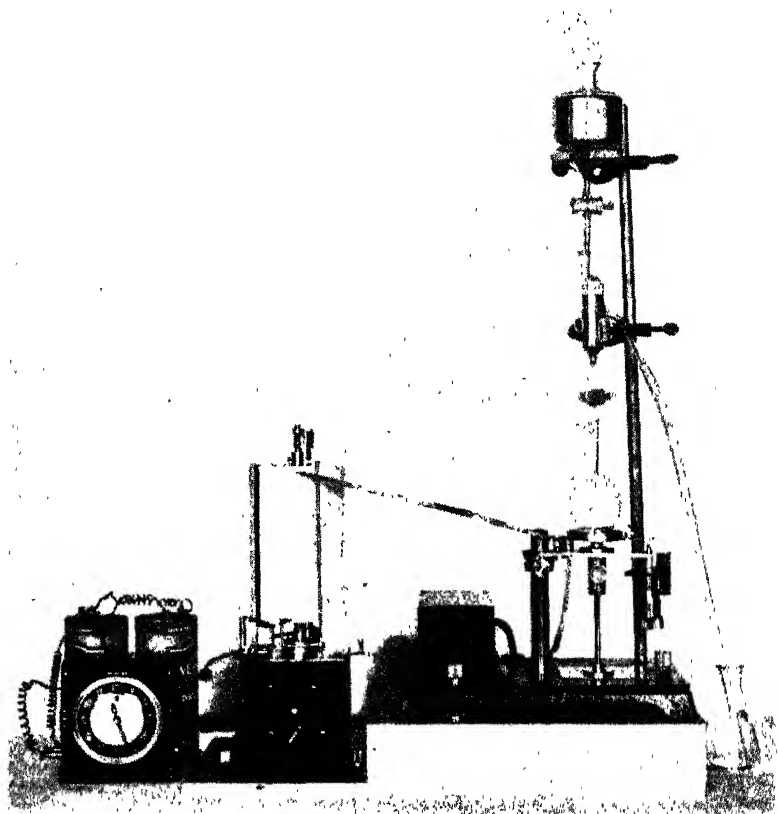


Fig. 3. Recording tenderness tester.

*Description of recording tenderness tester.*—The apparatus as finally developed is illustrated in Figure 3 and certain details in Figures 4 and 5. It consists essentially of a plunger terminating in a circular brass disc 30.5 mm. in diameter and fitted with a platform to hold a 125 ml. flask. The total weight of this assembly, including the flask, is approximately 160 g. Means are provided for loading with mercury at a constant rate, and the compression characteristics of the sample may be measured in terms of weight of mercury with the aid of a micrometer device, or a record may be obtained upon a slow-speed kymograph chart.

The main assembly is mounted on a stout pillar and is so located that the recording pen is approximately  $\frac{3}{4}$  inch below the top of the chart when the disc is in contact with the base of the instrument. This position is designated as the zero point and corresponds to a micrometer

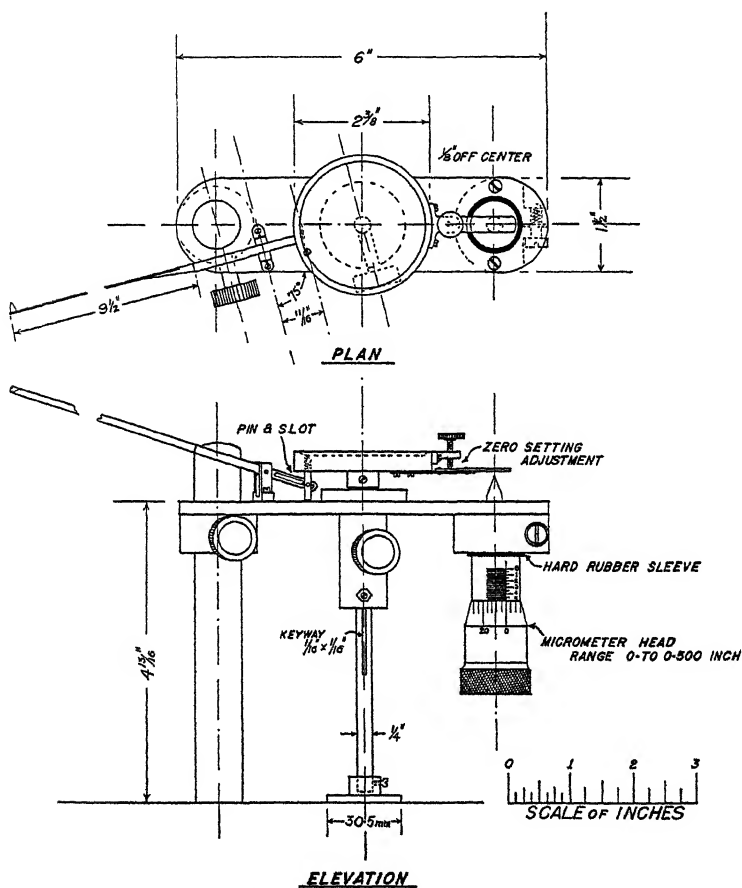


Fig. 4. Recording tenderness tester; detail of main assembly.

setting of .500 inch. The micrometer head is insulated from the remainder of the assembly, and an exact indication of the zero point is secured by means of an electrical contact between the micrometer spindle and a metal strip attached to the platform; this contact may be made to actuate a buzzer or signal light as desired. Setting of this zero is facilitated by mounting the contact strip on the end of a strip of spring bronze, which is raised or lowered by means of a fine thread

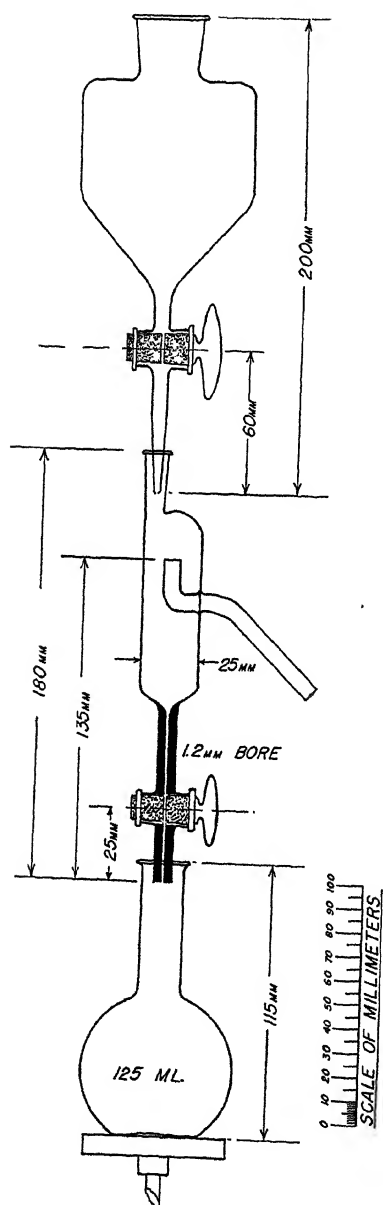


Fig. 5. Recording tenderness tester; detail of constant-head mercury delivery device.

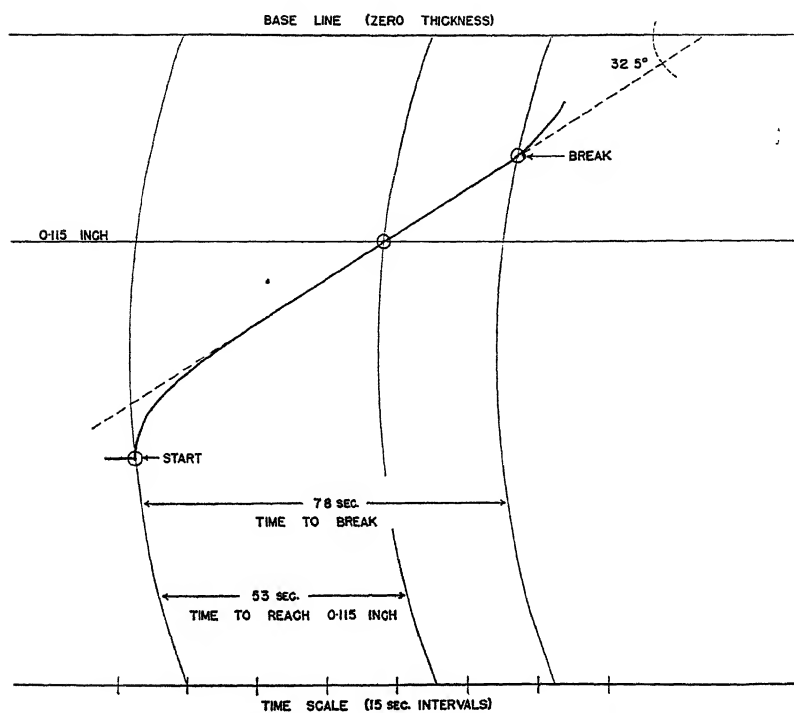


Fig. 6. Reproduction of tenderness test chart illustrating the measurements employed for evaluation.

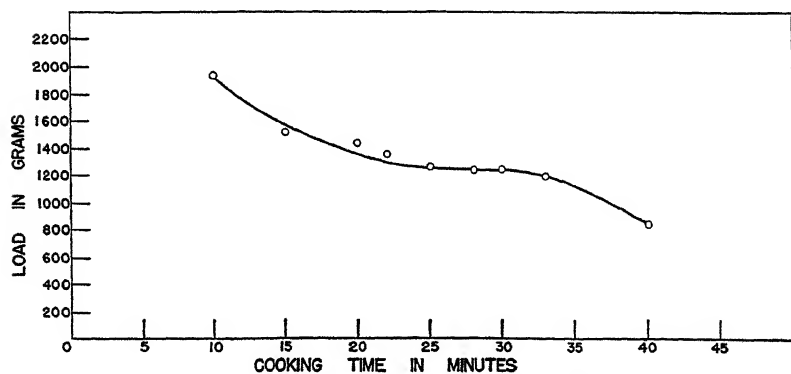


Fig. 7. Graph showing relation between cooking time and load required to compress the cooked macaroni to 25% of the original thickness.

screw. The micrometer device is employed to measure the original thickness of a sample and can then be set to indicate when compression has proceeded to a specified fraction of this amount; alternatively it may be used to establish a thickness scale upon the kymograph chart. This scale is then transferred to a strip of celluloid and used in measuring and interpreting charts obtained with the recording device.

For recording purposes, the vertical motion of the plunger is multiplied by means of a long lever arm coupled to the platform by means of the pin-and-slot device illustrated in Figure 4. When properly fitted, this system is practically free from backlash and permits of establishing a linear thickness scale upon the chart. In order to prevent turning of the platform with consequent disengagement of the pin from the slot, the plunger is key-way cut and a key fitted into the bearing. A lock-screw is also fitted enabling the system to be temporarily held in a raised position.

Recording is accomplished upon a kymograph drum 4 inches in diameter by  $6\frac{1}{4}$  inches in height, rotating at a speed of one revolution in approximately 11 minutes. The drive is from the hour spindle of a spring-wound clock suitably geared up. Unless very carefully constructed with specially cut gears, such a system possesses considerable backlash, and a uniform time scale cannot be established. For this reason, the kymograph has been fitted with a time-marking device actuated from a modified Telechron clock marking every 15 seconds. A synchronous motor drive would undoubtedly be superior to the clock-work arrangement described and might eliminate the necessity of an independent time-marking system.

Load is applied to the sample at a constant rate of approximately 12 g. per second, by means of mercury delivered from the constant-head device illustrated in Figure 5. In this apparatus, mercury flows into the constant-level vessel at a rate slightly in excess of its delivery to the loading flask, the surplus overflowing into a receiver. With this arrangement the head may be maintained constant within 1 to 2 mm. The orifice of the delivery tube is adjusted to deliver 57 c.c. per minute. Clean, redistilled mercury is employed and the delivery rate checked frequently, as it tends to slow down with time due to surface oxidation. When it has fallen to 55 c.c. per minute, the mercury is removed and cleansed by spraying through dilute nitric acid; the apparatus is also cleaned with the same solvent.

A typical record obtained with the tenderness tester is illustrated in Figure 6, together with details of the various characteristics of the curves that have been found most valuable in recording and interpreting the results. The best single index appears to be the time from the start of application of load to the break point. (This value can be expressed

as actual load, because the rate of application is constant; in view of the magnitude of the internal variability, however, no useful purpose could be served by such twelve-fold increase in these values.) The second value recorded is the time required to compress the sample to an arbitrary thickness of 0.115 inch. This point was selected because in the majority of cases it falls in the linear portion of the record and definitely below the break-point. The third value employed is the angle made between a prolongation of the linear portion of the curve and the so-called base line. A fourth value is secured by computing the ratio of "time to reach 0.115 inch" to "time to break." From these values a single-figure tenderness score is tentatively computed by the following formula:

$$\text{Tenderness score} = \text{time to break} + \text{angle} + (\text{ratio} \times 10).$$

*Details of cooking testing procedure.*—A high-temperature thermostat is employed for the actual cooking and is so adjusted that the water temperature in the beakers falls between 95.5° and 96.0° C. If oil is employed in the bath, a temperature of 105° to 106° C. is required; if, however, ethylene glycol (commercial Prestone) is used, a temperature of only 101.0° to 101.5° C. is required. A 500-c.c. tall-form lipless beaker is placed in the bath and 250 c.c. of distilled water, previously heated to approximately 95° C., is added. The beaker is covered with a watch glass and allowed to remain until the temperature reaches 95.5° to 96.0° C. A 25-g. sample of macaroni is introduced and thoroughly stirred. Cooking is continued for exactly 30 minutes, with stirring at 10-minute intervals. The beaker is then removed from the bath and the macaroni drained in a tared basket for two minutes, weighed, transferred to a beaker, and washed three times with cold tap water.

The sample is stored under tap water until required for the tenderness measurements which are made upon five strands selected at random, drained and placed on filter paper before locating under the plunger of the apparatus.

The additional values detailed by Borasio (1935) are outlined below.

*Volume of dry macaroni.*—This determination is conducted with a 10-g. sample and a small volumeter consisting of a 50-c.c. Erlenmeyer flask fitted with a ground-glass joint and a measuring tube graduated from 0 to 10 c.c. in 1/20 c.c. High-boiling petroleum naphtha is employed as the displacing liquid since water might introduce appreciable errors due to swelling during the determination. Borasio used water and apparently determined the dry volume on the cooking test sample; in our experience this prior wetting introduces serious irregularities into the tenderness results.



*Water absorption.*—This value is computed from the increase in weight upon cooking as outlined above.

*Volume increase with cooking.*—Originally, the measurement of wet volume was conducted according to the procedure outlined by Borasio, with a specially built volumeter. Examination of a large number of results, however, indicated that a very close relation existed between wet weight and volume, and statistical analysis of these data showed a correlation of .984, a value sufficiently high to permit of accurate prediction of the latter from the former by the following formula:

$$\text{Volume of cooked macaroni} = -8.81 + 1.0085 \times \text{net weight.}$$

The volume increase may be obtained by relating the wet volume to the dry volume; this latter value, however, has been found to vary only within a very narrow range, and for this reason it seemed unnecessary to carry this phase of the testing beyond the determination of water absorption.

*Residue.*—The drainings from the cooked sample are cooled and made up to 200 c.c. A 50-c.c. aliquot is transferred to a weighed 100-c.c. beaker evaporated to dryness on the steam bath and dried in a 130° C. air oven for 1 hour. If the presence of added salt is indicated, a correction must be made by ashing an aliquot of the residue and determining the chlorine content.

*Notes on the test.*—In the above description of the testing procedure, a cooking time of 30 minutes is specified; selection of this time was based on the cooking-curve data obtained in the preliminary studies. A typical curve of this kind is illustrated in Figure 7 and indicates the existence of an optimum tenderness region falling between 25 and 30 minutes of cooking. With 22½ minutes or less, the material would appear to be definitely on the "tough" side, and beyond 32½ minutes an irregular tendency towards excessive softness is noted. The existence of such a flat region in the cooking curve was confirmed by tests conducted at a later date, employing the recording instrument; data from a study of this type are presented in Table IV. It is of interest to note, however, that while the tenderness score indicates a leveling out in the 25- to 30-minute region, absorption and disintegration proceed at a fairly uniform rate throughout the whole period. As yet, insufficient results are available to state definitely whether or not this optimum cooking time varies greatly from sample to sample; the general trend of the evidence so far accumulated, however, indicates that for the majority of samples it falls between 25 and 30 minutes and a 30-minute cooking time has been employed in all our studies to date.

The effects of added salt represent an additional complication. As

TABLE IV  
EFFECT OF TIME OF COOKING UPON TENDERNESS SCORE, ABSORPTION,  
AND DISINTEGRATION

Time of cooking	A" Time to break	"B" Time to reach 0.115 inch	Ratio "A" to "B"	Angle	Single figure tenderness score	Absorption	Residue
<i>Min.</i>	<i>Sec.</i>	<i>Sec.</i>		<i>Deg.</i>		<i>%</i>	<i>%</i>
20.0	98	48	2.02	24.8	143.0	256	4.27
22.5	94	62	1.51	24.6	133.7	284	4.60
25.0	87	59	1.47	26.3	128.0	300	4.64
27.5	82	55	1.49	27.5	124.4	320	4.90
30.0	82	49	1.67	28.6	127.3	344	5.35
32.5	66	47	1.40	31.0	111.0	360	5.31
35.0	64	52	1.23	32.2	108.5	364	5.24
37.5	57	45	1.26	34.6	104.2	380	5.38
40.0	60	47	1.27	37.3	110.0	416	5.66

mentioned earlier, this was found to exert a marked softening effect, and data illustrating this are presented in Table V. In view of the fact that any reduction in tenderness might tend to minimize the spreads between samples, and also of the absence of salt in experimentally processed macaroni, the cooking tests have been conducted with dis-

TABLE V  
EFFECT OF ADDITIONS OF SODIUM CHLORIDE UPON THE COOKING  
CHARACTERISTICS OF MACARONI  
(Constant cooking time of 30 minutes)

Concentration of NaCl in cooking water	"A" Time to break	"B" Time to reach 0.115 inch	Ratio "A" to "B"	Angle	Single figure tenderness score	Absorption
<i>%</i>	<i>Sec.</i>	<i>Sec.</i>		<i>Deg.</i>		<i>%</i>
0.0	63	31	2.03	42.3	125.6	308
0.2	57	28	2.04	41.7	119.1	316
0.4	57	30	1.90	37.9	113.9	320
0.6	47	33	1.42	43.3	104.5	322
0.8	40	32	1.25	38.7	91.2	328
1.0	40	27	1.48	40.5	95.3	332

tilled water. Owing to the presence of varying quantities of added salt in commercial macaroni, the comparative tenderness scores would not necessarily indicate the relative inherent cooking properties of the pastes themselves; with this class of material it might be desirable to cook in a sufficiently high salt concentration to minimize the effect of variable salt content in the macaroni.

The replicability of the tenderness measurements between cookings is in the order of 4 to 8 units of tenderness score. The method has been applied successfully to a number of problems under investigation in this laboratory, however, and within several hundred tests upon macaroni processed from durum semolina the following range of values has been observed:

	Minimum	Maximum
Tenderness score	85.2	186.5
"Dry" volume, c.c. per 100 g. macaroni	69.6	73.2
Absorption, %	264.0	328.0
Wet volume, c.c. (computed) per 100 g. "dry" macaroni	358.3	432.8
Volume increase on cooking, times original volume	5.14	6.00
Residue, %	4.64	7.16

On the basis of these tests, a tentative scale of tenderness score values has been worked out as follows:

Soft.....	Tenderness score below 100
Slightly soft.....	Tenderness score 100-114
Normal.....	Tenderness score 115-129
Slightly tough.....	Tenderness score 130-144
Tough.....	Tenderness score 145-159
Very tough.....	Tenderness score over 160

It is very probable that the "slightly soft" and "slightly tough" groups fall in the category of satisfactory commercial tenderness, but more extensive studies, particularly with a wider range of commercial samples, are required before definite limits can be postulated.

### Discussion

In the work reported, the principal object has been the development of methods and apparatus for the quantitative measurement of the factors associated with macaroni quality. These factors have been classified into three major groups, namely color, mechanical strength, and cooking characteristics. Color measurement has been dealt with rather briefly, as the methods are fairly well known and the further extension of this phase of macaroni testing awaits the development of more suitable color-analyzing equipment. In connection with the cooking tests, all the data obtained so far have been for a single size of macaroni<sup>3</sup> and any departure from this class of material would undoubtedly affect the results. This criticism does not invalidate the utility of the method, however, and it is entirely possible that some means may be devised for relating the results obtained with different classes of material.

### Summary

The term "quality" as applied to macaroni products is discussed and the factors associated with desirable commercial characteristics are detailed.

Various methods of measuring color are described, suitable Munsell discs for matching macaroni products are listed, and formulae for calculating single-figure color scores from both Munsell and Wallace and Tiernan disc results are presented.

An instrument for measuring transverse breaking strength is described. The variability of the test is rather high; this appears to be associated more with variations in internal structure than with differences in wall thickness and diameter. The breaking strength of commercial macaroni is substantially greater than that of experimentally processed material of similar size, indicating that breaking strength is influenced by processing conditions. A relation between protein content and breaking strength is indicated but where varietal comparisons are involved, variations in other factors, probably associated with protein quality, obscure this relation.

The development of a standard cooking test is outlined, a recording instrument for measuring the tenderness of the cooked macaroni described, and a method for computing a tenderness score presented. The accuracy of the test is in the order of 4 to 8 units of tenderness score and a range of from approximately 85 to 186 units has been found for macaroni processed from durum semolina. Cooking in the presence of salt produces a pronounced softening effect.

Methods for determining dry volume, water absorption, increase in volume, and extent of disintegration upon cooking are also detailed.

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# THE WHEAT-MEAL-TIME-FERMENTATION TEST. II. EFFECT OF PROTEASES, PROTEASE ACTI- VATORS, AND PROTEASE INHIBITORS<sup>1</sup>

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In a previous paper<sup>4</sup> it was shown that the results in the wheat-meal-time-fermentation test are materially affected by fineness of grinding, length of storage after grinding, addition of ground bran or shorts, pumice stone, N-caproic and pelargonic acids, and by carbon dioxide dissolved in the water on which the doughball floats. Lecithin, oleic acid, paper pulp, and alundum had little effect on "time"<sup>5</sup> and no consistent effects were obtained from the use of  $\text{KBrO}_3$  and  $\text{KClO}_3$ .

It is not a simple matter to evaluate the "time" test as a quality measure. Spring wheats as a rule have a long "time," most hard winter wheats have a medium "time," a few have a long "time," and soft wheats as a rule have a short "time." Thus within certain limits it may be said that the test will differentiate varieties as strong, medium, and weak, using these terms not as indicating superiority or inferiority, but rather as adaptation for certain types of baking. One of the chief merits of the test is the small sample required and the comparative simplicity of the apparatus. This has made it most useful to plant breeders as it enables them to differentiate among hybrids and selections much earlier in their program than is possible with tests requiring larger amounts of grain. Although the relationship of "time" to baking results and other estimates of quality has not been clearly shown, there seems to be little doubt that it is a varietal quality factor particularly in the instance of crosses between long "time" and short "time" parents.

The method of mixing the meal, the technique of handling the doughballs, and the importance of the temperature control were discussed in the previous paper. Further experiments have shown that for grinding to pass a  $\frac{1}{2}$ -mm. sieve a small Jacobson hammer mill was more satisfactory than a burr mill. This hammer mill grinds more rapidly than the well known Wiley mill and is much more convenient to clean between samples. Since the particles pass the screen as soon as they have

<sup>1</sup> Much of the data in this paper is taken from a thesis presented to the graduate faculty of Kansas State College by Mr. F. T. Dines in partial fulfillment of the degree of Master of Science.

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<sup>3</sup> Contribution No. 57, Department of Milling Industry.

<sup>4</sup> C. O. Swanson, Factors which influence the results in the wheat-meal-time-fermentation tests, *Cereal Chem.* 14: 419-433, 1937.

<sup>5</sup> "Time" in this paper means the minutes from the moment the doughball was placed in the water until disintegration is observed on its underside while floating, and hence is used instead of the longer name for this test.

come to the required degree of fineness and cannot get away from the action of the hammers until this condition exists, a meal of fairly uniform granulation is produced. That the mechanical impact on the kernels from hard wheat would produce meal somewhat different in characteristics from the soft-wheat meal is no doubt true, but, as far as known, this in itself does not significantly influence the "time."

The technique of the test lends itself to the investigation of several problems. One of the most important of these has to do with the factors which influence the "time" and the present study was directed toward a partial solution of that problem. It had been observed that the time was influenced by proteases and their inhibitors. For this study it was desirable to use a normally long "time" wheat in comparison with a normally short "time" wheat. Tenmarq, a hard winter wheat, was used to represent the former and Clarkan, a soft winter wheat, the latter. Averages of a large number of determinations made on the meals, were 114 minutes for Tenmarq "time" and 40 minutes for Clarkan "time." Hence these two wheats were well adapted for this study because they give a wide range in "time" and they are also very different in other quality characteristics. Tests were also made on a number of other varieties and crosses as will appear in the tables which follow.

The proteases tried were pepsin, trypsin, and papain. The activator used was anhydrous cysteine-monohydrochloride. The inhibitors were potassium bromate and ascorbic acid, also known as cebione. These substances were usually added in water solutions by means of a syringe to the meal or flour while being mixed in the small mixer. The amounts given in the various tables represent what was added to the 15 g. of meal or flour.

### **Effect of the Amount of Pepsin on the "Time" of Tenmarq and Clarkan**

The data in Table I show the effects of different amounts of pepsin added to the meals of Tenmarq and Clarkan. The decreases in minutes due to pepsin are very small for Clarkan in amounts greater than 1.0 mg. and for lesser amounts the results indicate no consistent effect. For Tenmarq progressively greater decreases occur in "time" with increasing amounts of pepsin from 0.1 mg. to 1.5 mg. Larger dosages of pepsin do not give any significantly greater effect. The data indicate that 2 mg. of pepsin produces about the maximum effects; hence this amount was used in most of the subsequent work with the proteases.

It is evident that the behavior of Tenmarq is very different from that of Clarkan. The latter variety appears to be almost insensitive to

TABLE I  
EFFECTS OF VARYING AMOUNTS OF PEPsin ON THE "TIME" OF  
TENMARQ AND CLARKAN

Pepsin added	Tenmarq		Clarkan	
	Total	Decrease	Total	Decrease
<i>Mg.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
None (Av. 2 checks)	123	—	45	—
0.1	118	5	47	+2
0.2	108	15	45	0
0.3	103	20	54	+9
0.4	87	36	47	+2
0.5	82	41	41	4
1.0	69	54	46	+1
1.25	61	62	43	2
1.50	53	70	43	2
1.75	55	72	43	2
2.00	51	76	40	5
2.25	46	81	45	5
2.50	49	78	41	4

pepsin, while Tenmarq seems to be very sensitive because it drops very rapidly from a long initial "time" to almost as short a "time" as Clarkan. The decreasing magnitude in the amount of change for Tenmarq with the larger amounts of pepsin suggests that there is an irreducible minimum time below which not much reduction can be expected from the action of the protease. This appears to be similar to the limits in the reduction of loaf volume.

### Results with Trypsin and Papain

Trypsin and papain were tried in the same manner as pepsin and the results obtained with the various amounts are given in Table II.

There seems to be very little difference in effectiveness of trypsin and papain in decreasing the "time" of Tenmarq and for amounts larger than 1.0 mg. they compare closely to pepsin. For small amounts

TABLE II  
EFFECTS OF VARYING AMOUNTS OF TRYPSIN AND PAPAIN ON THE  
"TIME" OF TENMARQ AND CLARKAN

Amount added	Tenmarq				Clarkan			
	Trypsin		Papain		Trypsin		Papain	
	Total	Decrease	Total	Decrease	Total	Decrease	Total	Decrease
<i>Mg.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
None	108	—	108	—	37	—	37	—
0.5	58	50	65	43	35	2	37	0
1.0	38	70	35	73	36	1	37	0
1.5	39	69	37	71	35	2	32	3
2.0	40	68	33	75	37	0	30	7
2.5	36	72	30	68	36	1	30	7

such as 0.5 mg. and 1.0 mg. these two proteases produce greater decreases than pepsin. The effect on Clarkan was negligible for trypsin and very small for papain. Since there seemed to be no particular advantage in either trypsin or papain over pepsin in this investigation the latter was generally used.

### Effect of Pepsin on the "Time" of Hard, Semihard, and Soft Wheats

The large decrease in "time" due to the effect of pepsin on the hard wheat Tenmarq, as compared with the small effect on the soft wheat Clarkan, suggested the use of this protease on a number of hard, semihard, and soft-wheat varieties with 2 mg. for each 15 g. of meal. The data obtained are given in Table III.

TABLE III  
EFFECT OF PEPSIN ON THE "TIME" OF HARD, SEMIHARD AND SOFT WHEATS

Variety	Treatment		
	No pepsin	2 mg. pepsin	Decrease
	Min.	Min.	Min.
<i>Hard wheats</i>			
Oro x Tenmarq, Ks. 2729	130	65	65
Oro x Tenmarq, Ks. 2728	128	60	68
Oro	126	43	83
Tenmarq, Ks. 514	105	44	61
Cheyenne	94	42	52
Cheyenne sel., C.I. 11666	91	44	47
Turkey sel., C.I. 10094	90	53	37
Kanhull	88	42	46
Quivira	66	34	32
Kanred	51	39	12
Blackhull	51	39	12
Early Blackhull	47	37	10
Turkey	40	29	11
Chiefkan	37	30	7
Superhard	33	26	7
<i>Semihard wheats</i>			
Denton	108	49	59
Minturki	84	44	40
Kawvale	46	33	13
Iobred	41	31	10
<i>Soft wheats</i>			
Clarkan	39	37	2
Bald Rock	38	39	1+
Mo. Early Premium	33	32	1
Michigan Amber	33	34	1+
Harvest Queen	32	32	0
Red Rock	30	30	0
Mediterranean sel. C.I. 11567	30	28	2
Fulcaster	29	26	3
Currell	27	27	0
Michigan Wonder	27	28	1+



The variations in the data for the soft wheats are within the experimental error and hence it appears that pepsin produces no definite decrease. The hard wheats show a very large variation in the effect of pepsin in decreasing the time. Some hard wheats were affected comparatively little. As a rule the shorter the time of the hard wheats the less the effect of pepsin. This same statement may also be made for the semihard wheats. The implication of this is that on wheats which have a short "time," whether hard or soft, the effect of pepsin will be slight, and that on the hard wheats the decrease due to pepsin will be greater on long "time" than on short "time" wheats. Thus the nearer the "time" of the wheat is to the irreducible limit, the less the reduction possible with the use of a protease.

The "time" was determined on a series of flours milled from samples grown in the wheat-breeding nursery. The results are given in Table IV. The "time" on flours has been found as a rule to be

TABLE IV  
EFFECT OF PEPSIN ON THE "TIME" OF FLOUR FROM HYBRID WHEATS

Variety	Treatment		
	No pepsin	2 mg. pepsin	Decrease
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Oro x Tenmarq Ks. 2736	220	121	99
Fulhard x Kawvale, Ks. sel. 344154	197	127	70
Tenmarq Ks. 514	189	142	47
Oro x Tenmarq, Ks. sel. 343273	179	123	56
Kanred x Hard Federation, Ks. sel. 316063	175	121	54
Early Blackhull x Tenmarq, Ks. 2739	170	123	47
Oro x Tenmarq, Ks. sel. 363595	158	117	41
Oro x Tenmarq, Ks. sel. 363594	155	95	60
Oro x Tenmarq, Ks. sel. 363602	154	116	38
Oro x Tenmarq, Ks. 2730	142	115	27
Oro x Tenmarq, Ks. sel. 343638	139	110	29
Kanred x Kawvale, Ks. sel. J34584	139	113	26
Kanred x Marquis, Ks. sel. 285116	138	99	39
Oro x Tenmarq, Ks. sel. 343249	135	122	13
Tenmarq x Kawvale, Ks. sel. 33FN499	131	104	27
Kanred x Marquis, Ks. sel. 326795	127	100	27
Iobred x Kawvale, La. 35-95	127	83	44
Tenmarq x Kawvale, Ks. 2735	123	102	21

considerably longer than on meal. The decreases in "time" show considerable variation among the flours similar to that on the meals given in Table III. Direct comparisons cannot be made of the data in Tables III and IV because the meals and the flours were not from the same wheats.

### Effect of Protease Activators

If the short "time" on Clarkan is due to an active protease, and if the long time on Tenmarq is due to the inactivity of the protease, the "time" on Tenmarq should be considerably shortened by a protease activator while the same activator would have little effect on Clarkan. The data in Table V bear out this supposition. The protease activator has a definite effect in decreasing the time on Tenmarq, but the magnitude of the decrease is less than with pepsin (Table I). On Clarkan the effect on "time" was probably not significant. It is very evident that the activator affects these two wheats very differently. If the averages of the checks, 114 for Tenmarq and 40 for Clarkan, were used for comparison, the data would show still larger differences between these two wheats. There is then an indication that the long "time" on Tenmarq has some connection with an inactive protease, since the presence of an activator does shorten the "time" but on Clarkan, in which the protease is apparently already active, the activator has little effect.

TABLE V  
EFFECT OF A PROTEASE ACTIVATOR

Cysteine	Tenmarq		Clarkan	
	Total	Decrease	Total	Decrease
<i>Mg.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
None	110	—	36	—
0.5	107	3	43	7+
1.0	100	10	39	3+
1.5	76	34	42	6+
2.0	64	46	36	0
2.5	55	55	43	7+

### Combined Effects of a Protease and an Activator

The cysteine-monohydrochloride was used in gradually increasing amounts on Tenmarq and Clarkan with 0.5 mg. papain. The results obtained are given in Table VI.

It is very evident that the 0.5 mg. papain used in connection with the cysteine produced distinctly larger effects than the cysteine alone or even pepsin alone (Table II). This may be significant with Clarkan, in which the activator alone produced small increase in "time" (Table V), but in combination with the papain the decrease is small.

Papain alone (0.5 mg.) produced no greater effect on Tenmarq than 0.5 mg. pepsin, but 0.5 mg. papain in combination with 2.0 mg. pepsin decreased the "time" to 35 minutes, whereas 2.0 mg. papain

TABLE VI  
EFFECTS OF PAPAIN AND CYSTEINE

Amounts used		Tenmarq		Clarkan	
Papain	Cysteine	Total	Decrease	Total	Decrease
<i>Mg.</i>	<i>Mg.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
0.0	0.0	115	—	40	—
0.5	0.0	70	45	40	0
0.5	0.5	62	53	41	+1
0.5	1.0	58	57	41	+1
0.5	1.5	45	70	33	7
0.5	2.0	35	80	34	6
0.5	2.5	40	75	34	6
0.5	3.0	34	80	34	6

decreased the "time" only to 51 minutes (Table I). Thus the activator in combination with the protease produced greater decreases than when the protease was used alone. With 0.5 mg. papain plus 2.0 mg. activator the "time" on the two wheats was the same. This raises the question whether the differences in "time" obtained on varieties are due to variation in quality of proteins or to the state of the protease activity.

### Effects of Inhibitors

If the "time" is shortened by proteases and protease activators, then it should be lengthened by protease inhibitors. The inhibitors tried were potassium bromate and ascorbic acid (cebone). The effects of the bromate are given in Table VII and of cebione in Table VIII.

TABLE VII  
EFFECTS OF  $\text{KBrO}_3$  ON "TIME"

$\text{KBrO}_3$ used	Tenmarq		Clarkan	
	Total	Increase	Total	Increase
<i>Mg.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
None	106	—	36	—
0.25	141	35	40	4
0.50	155	49	43	7
0.75	188	82	46	10
1.00	222	116	49	13
1.25	223	117	55	19
1.50	247	141	52	16
1.75	244	138	52	16
2.00	233	127	48	12
2.25	277	171	55	19
2.50	250	144	50	14
2.75	279	173	54	18

TABLE VIII  
EFFECTS OF CEBIONE ON "TIME"

Cebione used	Tenmarq		Clarkan	
	Total	Increase	Total	Increase
<i>Mg.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
None	108	—	37	—
1.0	200+	92+	120	83
2.0	200+	92+	131	94
3.0	200+	92+	143	106

It is very evident that  $\text{KBrO}_3$  increases the "time" on both wheats but much more on Tenmarq than on Clarkan. The "time" for Tenmarq was increased 2.6 times the check while the "time" for Clarkan was 1.5 times the check. However the increments of the increases cease to be definite at 1.5 mg. for Tenmarq and 1.0 mg. for Clarkan. That is, beyond about 240 minutes for Tenmarq and about 50 minutes for Clarkan further fluctuations cease to have any meaning. This indicates there is a limit for maximum effects as well as for the minimum "time" already pointed out.

The cebione produced a much larger increase on Clarkan than did  $\text{KBrO}_3$ . The increase on Tenmarq was also large, but it was not possible to get a good endpoint, hence the time was marked simply 200+ and this was obtained with the 1.0 mg. of cebione, which compares in magnitude with the effect of 1.0 mg. of  $\text{KBrO}_3$ . The fact that one milligram of the protease inhibitor, ascorbic acid, makes the "time" on Clarkan as long as that of Tenmarq, points to the probability that the short time on Clarkan is due to the presence of an active protease.

### Effect of $\text{KBrO}_3$ and Pepsin

Pepsin was used alone and together with  $\text{KBrO}_3$  on Tenmarq and Clarkan. The results obtained are given in Table IX.

TABLE IX  
EFFECTS OF PEPSIN AND  $\text{KBrO}_3$

	Tenmarq		Clarkan	
	Total	Change	Total	Change
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
None	106	—	36	—
2 mg. pepsin	42	-64	40	+ 4
2 mg. $\text{KBrO}_3$	200+	+94	48	+12
2 mg. pepsin + 2 mg. $\text{KBrO}_3$	70	-36	49	+13

The four minutes' increase in Clarkan from 2 mg. of pepsin is within experimental error. The increase in "time" from 2 mg. of  $\text{KBrO}_3$  is very large on Tenmarq in comparison with the small increase on Clarkan. The reason for this is not apparent. The  $\text{KBrO}_3$  only partially inhibited the action of pepsin on Tenmarq. As was shown in Table I, pepsin had no effect on Clarkan; hence the combination of  $\text{KBrO}_3$  with pepsin would show the effect of only the former.

TABLE X  
EFFECT OF PEPSIN AND  $\text{KBrO}_3$  ON SPRING WHEATS

Variety	No pepsin	2 mg. pepsin	Decrease	2 mg. $\text{KBrO}_3$
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Garnet	228	145	83	250+
Apex	172	108	64	250+
Reward	171	105	66	250+
Thatcher	139	79	60	250+
Marquis	134	87	47	250+
Ceres	126	77	49	250+
Renown sel.	104	73	31	248+

Pepsin and  $\text{KBrO}_3$  were also tried on some additional spring wheats. The results obtained are given in Table X. The spring wheats behave in a manner similar to that of winter wheats in regard to the effect of pepsin. Those which have a long "time" show a greater decrease from the use of pepsin than those which have short "time." The proportional decrease, however, is about the same for both the long "time" and the short "time" wheats. The  $\text{KBrO}_3$  has a decided inhibiting effect on all the varieties, so much so that only on one was the exact time obtained.

### Summary and Discussion

The data presented in this paper show the following:

Pepsin decreases the "time" of Tenmarq to less than one-half that required without this protease. On Clarkan, a normally short "time" wheat, the effect is practically nil. Trypsin and papain in a limited trial had essentially the same effects as pepsin.

On a series of soft wheats whose "time" is normally short, there was no effect of pepsin. On a series of hard and semihard wheats the reduction in "time" due to pepsin was proportional to the length of "time" for untreated samples. That is, on the long "time" wheats the reduction was approximately one-half. This reduction decreased in proportion with shorter "times."

This behavior on long and short "time" wheats indicates that there is a limit to the possible reduction in "time." This is similar to reduction in loaf volume, which is not reduced beyond a certain amount, no matter how poor the flour.

Pepsin reduced the "time" on flours similarly to that of the wheat meal, but direct comparisons on meals and flours from the same wheats were not made.

The protease activator cysteine-monohydrochloride reduced the time on Tenmarq but not on Clarkan. This activator in combination with 0.5 mg. papain reduced the "time" on Tenmarq more than pepsin alone, or cysteine alone. The combination also made a considerable reduction in the "time" of Clarkan.

The protease inhibitor  $\text{KBrO}_3$  increased the "time" for Tenmarq 2.6 times and for Clarkan 1.5. The protease inhibitor ascorbic acid increased the "time" on Tenmarq apparently as much as did  $\text{KBrO}_3$ , and it made the "time" on Clarkan as long as the check "time" on Tenmarq.

Pepsin reduced the "time" on spring wheats similarly to its reduction on winter wheat.  $\text{KBrO}_3$  increased the "time" on these wheats beyond the limit of the accurate observation of the endpoint. That is,  $\text{KBrO}_3$  obliterated the "time" differences among the spring wheats.

While there appears considerable evidence to indicate that the length of "time" obtained on wheat varieties is due to the activity of proteases, this is not proved. Since the "time" on flour is longer than on the wheat meal, it would appear that the location of this protease is not in the endosperm. The disturbing fact is that, as has been shown in a previous paper and will be further presented in another paper, the addition of the bran material to the flour increases instead of shortens the "time." That there is also a gluten quality factor appears from the fact that the effects of the proteases and protease inhibitors were not the same for Tenmarq and Clarkan. Thus while these investigations give informative data, the real cause of differences in "time" on wheats is not clear but needs further investigation.

# A CONVENIENT APPARATUS FOR GAS PRODUCTION DETERMINATIONS BY THE BLISH METHOD<sup>1</sup>

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(Read at the Annual Meeting, May 1938)

It is evident from the comparisons made by Eva, Geddes, and Frisell (1937) of the three main types of apparatus for the measurement of gas production, that the pressure method (Blish, Sandstedt, and Astleford, 1932, and Sandstedt and Blish, 1934) is superior to the others in convenience, in the small quantity of material required, and in the low cost of the equipment. Landis (1934) used gauges to measure the pressures. It seemed that the convenience of gauges could be obtained and, at the same time, the cost of the apparatus reduced by the use of a single gauge to indicate the pressure in several fermentation vessels. This paper describes an apparatus constructed on this principle.

## Construction of Apparatus

The apparatus is divided into two sections, each having six units. In each section the fermentation vessels are connected through valves and a header, filled with tetralin, to a single gauge. Each section is supported on a light frame which permits raising and lowering into a constant-temperature water bath, 28 × 10 × 10 inches, to which the frames are attached. Figure 1 shows one section in the raised position, while the other is immersed in the bath. The frame consists of ¼-inch brass rod which slides in guides made of ¼-inch iron pipe. The apparatus is held in the raised position by spring catches engaging with slots in the rod. These catches may be disengaged by the little finger of the operator while the rest of the hand controls the lowering. The construction of the apparatus can be best described by dealing with each part separately, and referring to the diagrammatic drawing in Figure 2.

*Fermentation vessels.*—The vessels were made from 8-oz. De Vilbiss spray-paint cans. These are die-pressed aluminum cans with covers that can be clamped tight by means of thumb screws (*C*) and yokes (*A*) engaging with pins (*B*) on sides of the cans. They have a capacity of 305 c.c. and are very uniform in volume. The leather gaskets were replaced by rubber ones made from a black stock 1/16 inch thick, with hardness 43 (Shore Durometer Type A), fastened in place with Vultex paste. In order to ensure perfect circles, a brass die with two concentric cutting edges was made for cutting the gaskets. The diameters

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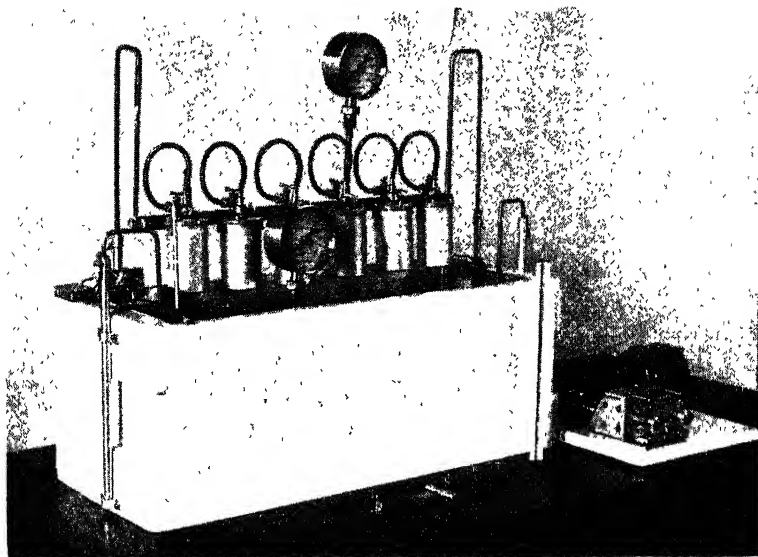


Fig. 1. Gas production apparatus.

of the cutting edges were slightly smaller than the desired size of the gasket to compensate for the stretching of the rubber. The rubber was laid on a cardboard pad and the die pressed through it in a hydraulic press.

The places where the pins (*B*) pass through the walls of the cans were made air-tight with De Khotinsky cement. The vents in the covers were similarly sealed.

The paint tube, which reaches to the bottom of the can, was sawed off. The connecting nut was removed and the inside of the delivery tube was reamed out to take a  $\frac{1}{4}$ -inch O.D. copper tube (*D*), which was soldered in place.

*Gauges.*—These are test-grade Bourdon tube gauges with an accuracy of 0.5%, graduated in millimeters of mercury to 1200 mm. The very small change in volume of a Bourdon tube with increasing pressure makes it possible to use a single gauge for several units as there is practically no movement of tetralin when the readings are taken. It is essential that high grade gauges be used, because cheaper ones have neither the accuracy nor sensitivity required.

*Header assembly.*—The header itself is made of heavy-wall brass tubing to permit threading in the other components. The ends are closed by screw plugs to which the supporting frame is fastened. Weatherhead needle valves No. 6855 are used to connect each can to the header. It is necessary to repack the glands carefully in order to



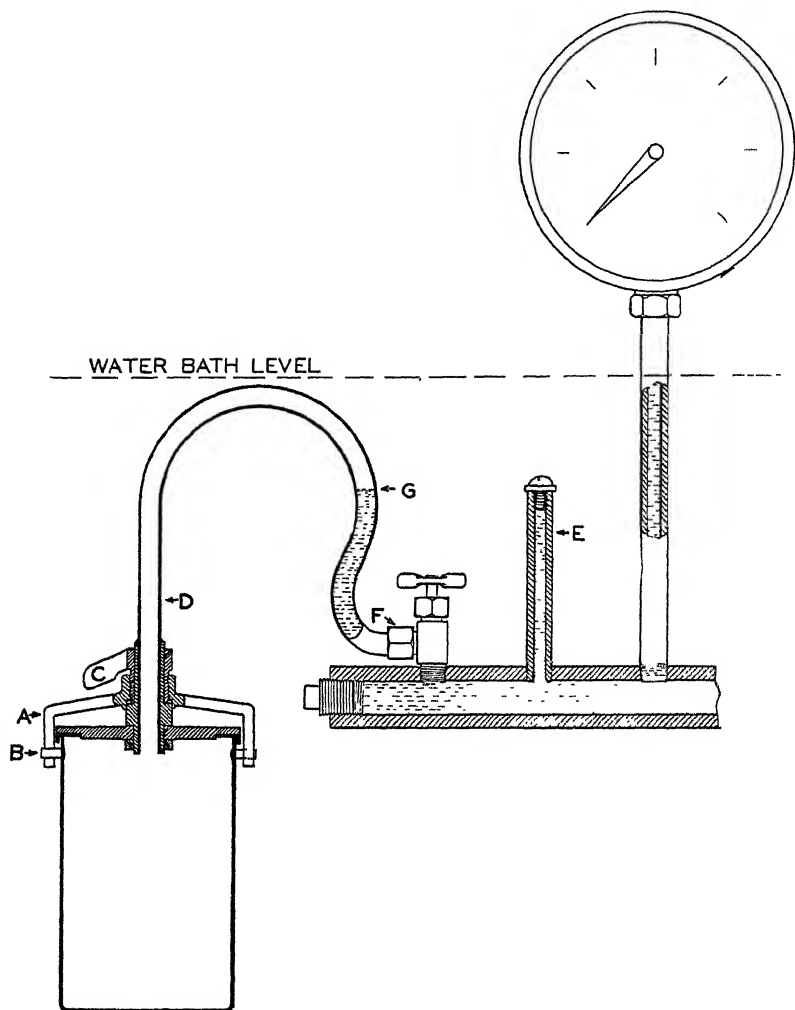


Fig. 2. Construction of apparatus.

prevent leakage under pressure. The header is provided with a tubule (*E*) with a gasketed screw-plug. This is used for filling the header. All the joints were soldered with the exception of that at *F*, which can be pulled tight. After assembly the whole apparatus was subjected to air pressure at 1000 mm. and was found to be gas tight.

Tetralin was selected as the liquid to fill the header because of its high fluidity and extremely low vapor pressure. Before introducing it into the system, the Bourdon tube was blocked in place to prevent

distortion. The valves were closed and the tetralin was run in under vacuum through the tubule (*E*), filling the header and Bourdon tube. The vacuum pump was then disconnected, the valves opened, and additional tetralin poured into the tubule until it overflowed, partially filling the tube to each fermentation vessel (to the level *G*). Finally the tubule was plugged and the pointer of the gauge was adjusted to the zero point as it was displaced slightly by the weight of the tetralin in the Bourdon tube.

### Operation

The fermentation vessels are clamped in position and the water bath is brought to temperature several hours before the determinations are to be started, in order that the entire apparatus may be at 30° C. As the samples are made ready, one section is raised out of the water, one of the vessels removed, the sample put in, the vessel again clamped in place and the section reimmersed. If it is desired to release the pressure at the end of five minutes, the section is raised and the clamping screw loosened and retightened. Readings are taken simply by opening the appropriate valve momentarily with the long handled wrench shown in front of the bath in Figure 1, and observing the gauge.

In order to prevent loss of tetralin, it is advisable to have all the valves closed when the fermentation vessels are being put on or taken off. The valves should be open when changes in temperature may take place, in order to avoid strain on the Bourdon tubes.

When the gas production of dough samples is being determined, the doughs are mixed in a miniature paddle-type mixer capable of mixing from 10 to 25 grams of flour, which was constructed in this laboratory. The paddles and bowl are made from the transparent plastic "Lucite" and have proved to be very satisfactory in operation. The mixer is shown beside the bath in Figure 1.

### Discussion

This apparatus has proved to be quite convenient in routine operation and fully as accurate as the single pressure meters fitted with manometers. In a special uniformity trial the standard error was 4.4 mm. and the variation determined from the differences between duplicates in ordinary routine determinations was S.E. = 6.6 mm. These standard errors compare favourably with the value (S.E. = 7.97) obtained by Eva, Geddes, and Frisell.

The use of the tetralin-filled header permits the use of expensive high-grade gauges without adding greatly to the expense per unit. The clamping arrangement is more convenient than the lock ring, which requires a wrench and a clamp to hold the cup for its operation. The thin pressed vessels are uniform in size and they quickly assume

the temperature of the bath. Pressed vessels can be made more cheaply in quantity than cast and machined ones, particularly when the material used is difficult to machine, as is the case with aluminum. No release valve was provided in this apparatus but bicycle-tire valves could readily be fitted to the cover of each vessel if desired. The out-of-pocket cost of the apparatus was approximately \$7.50 per unit.

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## THE UTILIZATION OF SOFT-WHEAT FLOUR

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(Read at the Annual Meeting, May 1938)

In considering the utilization of soft-wheat flours, it is found that they appear to fall in three general classifications, with possibly a fourth or miscellaneous group.

#### *Uses of Soft-Wheat Flour*

<i>Family</i>	<i>Cracker and biscuit</i>	<i>Commercial bakery</i>	<i>Miscellaneous</i>
Family bread	Cracker sponge	Cake	Starch
Biscuit	Cracker dough	Cookie	Soup thickenings
Self-rising	Pretzel	Sweet goods	
Cake	Cone	Doughnut	
Pastry	Cookie	Pie crust	
Pancake, etc.	Cake	Pastry	

In soft-wheat-producing sections of this country, a large amount of soft-wheat flour is used for family purposes. So, for our first class we have the family flours, which include family bread, general-purpose pastry, cake, biscuit, and self-rising flours. A second class of flours might well be called the cracker and biscuit flour group. Among this group will be found the cracker sponge, cracker dough, cookie, and flours suitable for pretzels and cones. In other words, these flours go into specialized bake shops. A third general class might be called the

commercial bake shop flours. Heading this group are cake flours, pastry, pie crust, yeast-raised sweet goods, such as sweet rolls and cinnamon rolls, and doughnut flours, etc. In the fourth or miscellaneous class are found flours which are used in the manufacture of various other products such as wheat starch, pastes, soup thickenings. This group does not account for a very large amount of flour going into commercial use, and will not be discussed farther in this paper. There is some overlapping in the groups outlined in the above classification; *e.g.* cake flour appears in all three classes. Pastry flours might also appear in all classes.

### Family Flours

Let us now take up each of these classes a little more in detail with a view to seeing how well they are standardized, and with reference to some of the problems connected with their utilization. The baking of bread in the home, as we know, is fast becoming a thing of the past, but throughout the rural communities and in the foreign sections of our large cities, the making of bread is not quite a lost art. Throughout the soft-wheat territory, because of price and the skill in its use, soft-wheat flour is the basic flour for home bread making. Because of wide variation in protein strength in different sections and variations from year to year in the quality and character of the protein, soft-wheat mills find it advantageous to blend in hard or spring wheat to maintain as nearly uniform a family bread flour as possible. From the analysis of a great many flours of this type we find the range of protein to be from 9.5 to 10.25%, with viscosities ranging from 90° to 150° MacM. The stronger blends are used in foreign sections of our larger cities, while the weaker blends find use in the rural communities and on the farms.

The general-purpose pastry flours cover a variety of uses, and include flours with a wide range in characteristics. They are, in the main, mostly straight-grade soft-wheat flours, milled and sold in the community producing the wheat. Their uses include family bread baking, all types of home pastry, hot breads, thickening, etc. There is no particular standard for this type of flour, ranging from soft white wheat flour in Michigan to the stronger flours of Illinois and Missouri. Special family cake flours, or packaged cake flours, have characteristics in common with the bake-shop cake flours and will be taken up under the commercial cake-flour group.

Biscuit flours and self-rising flours constitute a large portion of the flour sold in the South, and concerning them a great deal has been written. Our A.A.C.C. Committee on Biscuit and Self-Rising Flours has worked for a number of years on methods of testing and evaluating these flours. Most flours, whether hard or soft, when baked with the

*correct* formula, give satisfactory results. The confusion arising over the proper amount of shortening to use is due to the different types of flour employed. Soft-wheat flours require less shortening than hard-wheat flours, and often the failure of the housewife to obtain satisfactory results is due to her use of the wrong recipe. To err is human, and with self-rising flours in which two or more ingredients are added to a flour there is sometimes an error. The most common error is the leaving out of one of the necessary ingredients, or sometimes even a doubling up of one of the ingredients, improper mixing, etc. On the past year's crop with its rather high moisture content a great many mills experienced trouble with self-rising flour which contained too high a moisture content. This flour, when stored for some time, lost part of its leavening action, and in some extreme cases showed very little if any remaining gassing power. Too high a moisture content may result in shot balls, even with a prepared flour salt. In some tests conducted a few years ago in the laboratory we found that flours were perfectly safe under 13.5% moisture and there was very little, if any, trouble at 14.0%. But above this figure there was a loss of gas and shot balls formed. We were not using a prepared flour salt.

Too strong a bleach with chlorine or Beta-Chlora produces a lower-volume biscuit. We had one miller making a very short patent flour. He treated this flour to a pH of 5.20 and then added one-half percent of phosphate. Of course his object was to have a high-grade biscuit and cake flour, with the result that he fell somewhat short of either.

### Cracker and Biscuit Flours

We now come to the specialized bakery group, headed of course by the cracker and biscuit flours. This group constitutes a considerable portion of the commercially milled soft-wheat flours, and is probably on a more scientific basis than the other groups of flours, with possibly the exception of cake flours. The cracker flours are classed in two groups, the cracker sponge and the cracker dough flour. The cracker sponge flour must be of a type capable of withstanding a rather long fermentation, and at the end of this fermentation, varying from 18 to 22 hours, to be so conditioned that the resulting cracker is not too tough or too brittle. This flour is characterized in the laboratory by a live dough, the loaf showing good spring in the oven with a fairly definite break, and a clean shred. For this type of dough, the cracker baker usually wants an unbleached soft-wheat flour, well milled, with the low grade removed. Such flours are usually made from the stronger red wheats with a viscosity ranging from 55° to 65° MacM., although on previous crops viscosities from 70° to 80° were not uncommon. Viscosities above this figure usually indicate the likelihood of too heavy or too tough a cracker.

For the cracker dough, a soft-wheat flour, somewhat milder than the sponge flour but not as weak as a cookie flour, is required. This flour should be well milled, of about the same grade as the sponge flour, unbleached, and with a viscosity varying from 40° to 50° MacM. This type of flour may be made from the milder red wheats or from a blend of red and white wheat.

Some cracker bakers use only one type of flour for both the sponge and the dough. This flour should therefore border more closely on the cracker sponge type, and flours with viscosities between 50° and 55° MacM. work quite well for this class of bakers. The cracker baker has his difficulties from year to year, the same as the bread baker. This is due to the fact that there are some years in which the gluten is high, with strong characteristics, while in other years the gluten may be lower or of a much milder character.

To the cracker and biscuit trade, biscuits mean all sorts of fancy cakes and cookies, and vary from the size of a dime to that of a saucer. Some of these cookies are on the order of cakes. They rise and have a definite texture similar to cake. Others spread as they are baked, and have no definite texture. For the cake-type cookies, a good grade of soft-wheat flour, having mild gluten, softer than that of the cracker-dough flour, is used. This flour should be treated with Beta-Chloral or chlorine, much the same as cake flours. For the type of cookies which spread when baked, an unbleached flour gives the best results. In fact, if a chlorinated flour is used for such cookies they tend to rise instead of spread. Instead of having ginger snaps we have ginger cakes. In both these cookie flours a milder type of flour is used, and while no definite standards have been set up, it is found that a considerable amount of soft white wheat flour is used, as well as the milder-type red-wheat flours. The gluten content may vary from as low as 6.75% to 7.5%, with a viscosity range from 25° to 40° MacM.

The pretzel and cone flours coming under this classification are far from being standardized. However the late D. A. Coleman pointed out, in a talk before the American Millers Association at St. Louis in May, 1936, that a survey by the U. S. Department of Agriculture revealed that 98% of the flour used by pretzel bakers was either soft-wheat flour or blends in which soft-wheat flour predominated.

### Commercial Baking Flours

The use of soft-wheat flour has tended to remain somewhat at a standstill. With the advent of high-speed machinery and modern bakery practice, coupled with improved transportation, the small bakery in the soft-wheat territories has found it advantageous to swing to the

hard wheat flours for his bread baking. Nevertheless, during years of strong soft wheat, such as we had in 1934, considerable soft wheat was used in bread baking, because of a favorable price. However, for pastry, sweet goods, and cakes, the commercial baker has increased his use of soft-wheat flour.

For pastry and pie-crust use, a considerable amount of the lower-grade flour finds favor with the baker. The protein may be high, but it is of a mild quality and is economical on shortening. There are some who have treated the flour with Beta-Chlora or chlorine in order to reduce the gluten strength. This has a tendency to give a very thick mealy pie crust, having a light color, whereas the untreated lower-grade flours tend toward a thinner crust, somewhat flaky, and having a natural brown color. Aside from the lower-grade flours, the weak "starchy" flours are used very successfully by the pie baker. If we were to set a viscosity range for this class of flours, it would be from 25° to 35° MacM. In our laboratory experience we have found that an excess of water will invariably produce a tough crust. Apparently when too much moisture is added there is some development of the gluten in the mixing. In the official method (tentative) for testing pie flour (A.A.C.C., 1935) the formula calls for from 50% to 64% cold water. In testing soft-wheat flours for this purpose, I find from 35% to 40% absorption gives much better results. Also, this formula calls for 60% shortening, which tends towards too rich a crust with the majority of soft-wheat flours.

Probably the greatest increase in the use of soft-wheat flour by the commercial baker has been for the baking of cakes. This is partly due to the housewife's desire for more leisure time and to the improvement in the bakery cakes, which are now made on a more scientific basis than they were a few years ago. A high-grade cake flour must now meet certain specifications in order to produce a cake with a good volume and a silky texture and be capable of carrying a large amount of sugar. The proportion of flour used in cakes is much smaller than it is in any other class of baked goods, but this flour must be capable of doing its part. The flour content of some high-sugar-ratio cakes is as low as 28% of the total batter. The protein content may vary from year to year, but the average will be around 7.5% to 8.0%. This gluten should be of a natural, moderately mild quality, with a viscosity around 50° MacM. Some successful cake flours run somewhat higher than this, and on this year's crop many are running much lower, but some slight adjustment in the cake formula is necessary. While ash apparently has no effect on the cake-baking properties of a flour, it is indicative of good milling. Those streams which produce the best cake flours are naturally low in ash and as a result it is said that a cake flour should

be low in ash. Probably the majority of cake flours have an average ash content of from 0.32% to 0.34% on a 13.5% moisture basis.

One factor which seems to have received too little attention, and which has caused no little grief in the baking of cakes, is the moisture content of the flour. This has been particularly noticeable on this last year's soft-wheat crop (1937-38), which had an abnormally high moisture. This should be given consideration, especially in the high-sugar-ratio cakes, where the formula calls for a high percentage of added liquid. A high moisture in the flour may be just enough to unbalance the formula, causing the cakes to fall and produce very definite sugar lines. This is very plainly shown in Figure 1. This flour was received in the laboratory and baked with the results shown on the right. The analysis of this flour was very good, with the exception of the moisture content, which was 14.9%. This flour was then dried over night to a moisture of 12.6%, and re-baked the following day, with the results shown on the left. The high-moisture cake showed less volume, even before it fell, and a soggy sugar line through the center.



Fig. 1. Effect of excessive moisture on cake properties.

I believe the high-sugar-ratio cakes are more critical, or we might say have a smaller moisture tolerance than the older-type cakes. It might be well in the development of new formulas in the future if all the characteristics of flour were taken into consideration. One very important factor in cake flours is the proper treatment with Beta-Chloral or chlorine. The ideal treatment seems to be that producing a pH of 5.20. This of course may vary from 5.10 to 5.30. Less acidity tends towards coarser texture and smaller volume, with untreated flours giving the same results in high-ratio cakes as observed in the cake with too high a moisture content, namely falling in the center and having a pronounced soggy sugar line. Too strong a treatment, say with a pH below 4.75, tends toward lower cake volume and instability so far as the keeping qualities of the flour are concerned.

Even with fairly definite standards for cake flours available, those offered to the trade cover a wide range and cause no little confusion. The low-protein, low-viscosity white wheat cake flours are often sold



to a trade which has been using a much stronger flour made from some of the stronger red wheats, or *vice versa*. These same conditions in baker's cake flours also apply to the packaged cake flours used by the housewife.

In conclusion, it may be said that soft-wheat flours find their outlet in a multitude of products, and only in a few cases have fairly definite standards been set up. The setting up of more definite standards (keeping in mind the variation occurring in soft wheat from different sections from year to year) and the obtaining of the co-operation of the entire baking industry in conforming to these standards, will aid greatly in the milling of soft-wheat flour and encourage the use of these flours on a more extended scale.

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## THE TECHNIC OF PRODUCING A NEW SOFT WHEAT <sup>1</sup>

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(Read at the Annual Meeting, May 1938)

Three general methods are used in obtaining new wheat varieties, namely introduction, selection, and crossing. By introducing new strains from other states and countries and comparing them with known commercial varieties, new wheats may be found that are suitable and adapted for a particular locality. The variety Turkey, grown very extensively in the hard red winter wheat region (and introduced from Russia), illustrates this method of improvement. When the selection method is used, several hundred heads of various types are isolated from the best commercial varieties. Each is grown separately and tested in comparison with suitable standard varieties with the hope that some will be more desirable than those already grown. Trumbull, a soft winter wheat selected from Fultz, was developed by this method.

The introduction and selection methods have been used for many years with great success. Their use, however, is limited since most of the better varieties and selections already have been tested, and furthermore, one is limited to only those types produced by nature. If the types desired are not available it is up to the agronomist to create or "build up" such new varieties. This is done by crossing, the method most extensively used in developing new wheats.

Before taking up crossing as a method of developing new wheats,

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it seems desirable to emphasize several important concepts. First of all, to an agronomist, wheat characters are his units or "building blocks." He has many parental wheat varieties that possess one or more outstanding characters. The characters are analyzed into their individual components and the characteristics and behavior of each studied. These are then synthesized into new combinations or types as demanded by the grower and trade, just as the chemist selects his chemical elements, studies their properties and characteristics, and then synthesizes new compounds.

An analogy may be briefly drawn between the various parts of a house and wheat characters. Because of its importance in a new wheat, yielding ability may be thought of as the foundation. The windows represent the individual components of quality; the heating system indicates winter-hardiness; the siding, paint, and insulation, disease resistance, etc. Most of these characters are very important and must be considered in developing a new variety. A wheat possessing only high yielding ability, however, is as complete as a house with only the foundation. Obviously, all characters do not have the same relative importance, because of their nature or the conditions under which they are grown. For example it is immaterial whether a wheat possesses white or brown chaff, whereas it is imperative to have desirable winter-hardiness, high yielding ability, disease resistance, and suitable quality. Likewise, a variety grown in the north must possess greater winter-hardiness than one grown in the southern states.

In developing new varieties, therefore, an agronomist has specific objectives in mind. In the soft winter wheat region special emphasis is given to the combining of high yield with winter-hardiness and greater disease resistance, together with the suitable quality already found in these wheats. The crossing method is used in synthesizing these new combinations. Before making the cross, however, great care is taken in selecting the proper parents, since one or the other parent must have the characters that are to be combined. After suitable parents have been selected the cross is made. Figure 1 shows the floral organs of a single wheat flower.

In making the cross the head or spike is trimmed to about 16 or 18 flowers by removing the lower and upper spikelets. The anthers, while green, are removed from each flower by pulling them out with a pair of forceps. A glassine bag to prevent contamination of foreign pollen is then placed on the emasculated spike, and the female parent is properly labeled by means of a small tag. Pollination is usually done two or three days after emasculation. Anthers of the male or pollen parent, which are yellow and ready to dehisce pollen, are gathered in a small bottle or watch glass. The glassine bag is removed from the female

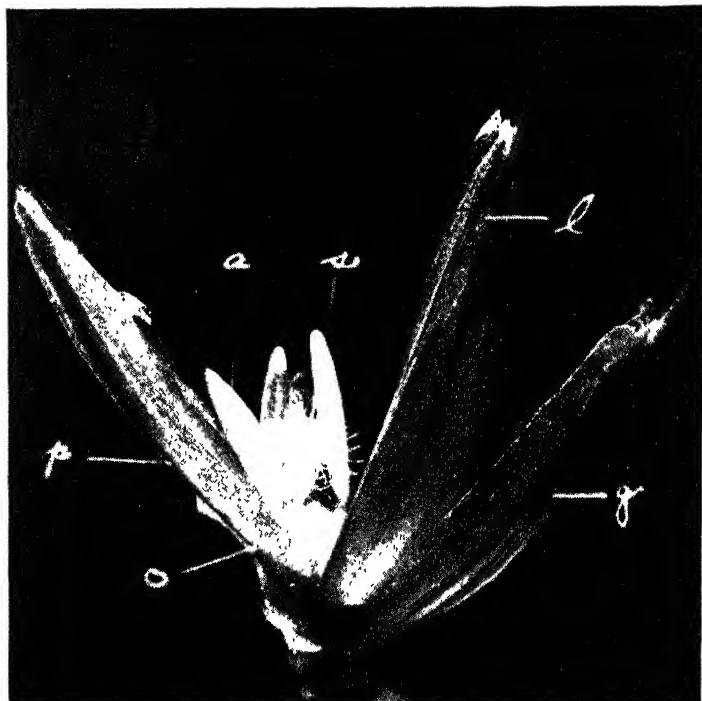


FIG. 1. Photomicrograph ( $\times 10$ ) illustrating the floral parts of a wheat flower: (a, anthers; s, stigma; o, ovary; l, lemma; p, palea; g, glume).

parent and the ripe anthers containing the pollen are placed on the stigma of each flower. The glassine bag is then replaced, the male parent recorded on the tag, and the bag left on the head until harvest. The cross presumably has been completed, and if successful, a few  $F_1$  grains should result.

When a cross between two varieties has been made and the grain grown for a few generations, many new types and combinations have been synthesized. The task of the agronomist then is to discover the desirable new combinations. This is done by rigid and systematic selection which includes both rejection and retention, since only a very few of the new strains from any one cross are found to be desirable. The progressive steps in breeding new wheats during the early segregating generations are briefly illustrated in Figure 2.

Starting, for example, with a single  $F_1$  cross obtained in 1930, a year elapses before  $F_1$  plants bearing a few hundred grains are produced. These grains are planted in the field by spacing them in rows. The following year each plant is examined and only the desirable types are continued in short rows as shown in Figure 3.

The plants in these rows in turn are examined and studied, and from the most promising ones, head selections are made for the following planting. This process of reselection is continued for six to eight years. This period is very important in a breeding program and

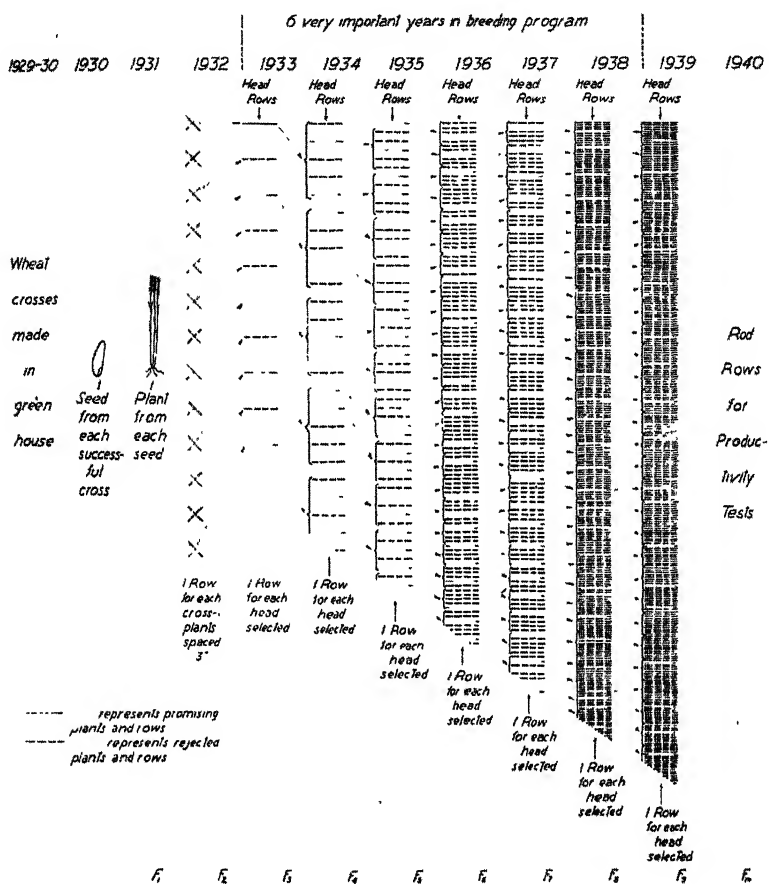


Fig. 2. Progressive steps in breeding new wheats during the early segregating generations.

the most difficult to the agronomist. Each year several thousand strains are grown, examined in the field, and the more desirable ones harvested. Each year several thousand samples, representing only 10 to 20 grams of wheat of each strain, are examined and tested for size, type, quality, etc. Simple characters, such as maturity, beards, shattering, strength of straw, height, type, etc., are readily recognized and are

used as a basis for selection. The more complicated characters, such as quality, winter-hardiness, and yield, cannot be recognized by merely observing the plants or the sample of grain. The agronomist therefore depends on specific tests or "performance tests" to guide him in the desired direction as to quality, winter-hardiness, and yield.



Fig. 3. Four-foot rows in which new selections are grown, reselected, and purified.

Obviously, the more simple characters that may be easily recognized are used at first as a basis of selection. As the work proceeds, each new combination that is selected is then subjected to numerous performance tests. The first of these is the quality test to ascertain milling and baking value. In Indiana three simple tests are used to measure the important components of quality, (*a*) the wheat-meal fermentation-time test as a measure of gluten strength, (*b*) the granulation test with which is obtained the degree of particle fineness, and (*c*) the carotenoid-pigment content to determine desirable color. Fortunately all three tests can be made in the early segregating generations, since only a small quantity of seed is required. Later, when sufficient grain is available milling and baking tests are performed on all strains before being released for commercial production.

All strains that appear satisfactory for the simple characters and meet the qualifications of a suitable soft wheat for pastry purposes, are then subjected to the winter-hardiness tests. This involves replicated field tests as well as growing seedlings from each strain under

field conditions and subjecting them to an "artificial winter" produced in a refrigeration chamber. Only those selections which meet these severe tests for cold resistance are retained.

Strains that have survived six to eight years of rigid selection and appear quite satisfactory and pure for the characters examined, are then advanced for yield trials in rod rows as shown in Figure 4.

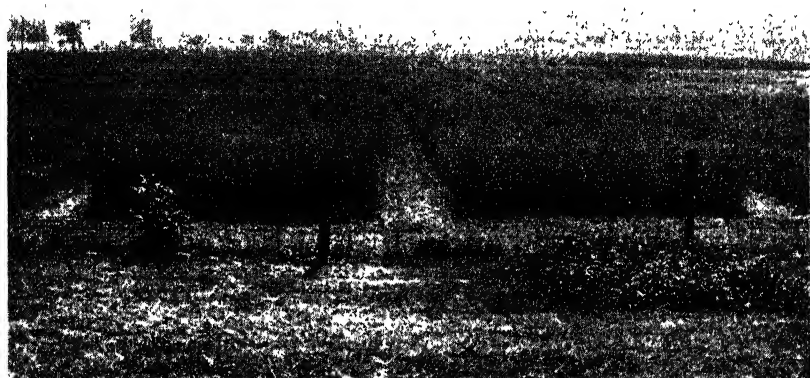


FIG. 4. Rod-row nursery in which yielding ability of new strains is determined.

Yield trials in rod rows are conducted over a period of four to five years in an effort to test seasonal, soil, and locality adaptation. Those strains that consistently demonstrate superiority in yielding ability, as well as quality, winter-hardiness, disease resistance, etc., are then advanced to multiplying plots and field test plots. The number of strains to reach this test, for obvious reasons, is very small. Those that do are tested not only at the central station but also at several outlying fields located throughout the state under different soil and climatic conditions. Check plots, seeded with standard varieties, are grown with these for the purpose of comparing yields and other agronomic and physiologic characters. In addition, one or two of the most promising strains are further tested in special disease and winter-hardiness nurseries located throughout the United States. These are conducted co-operatively between the United States Department of Agriculture, Bureau of Plant Industry, and State Agricultural Experiment Stations.

If a strain consistently demonstrates superiority during the 12 to 15 years of rigid and systematic testing, it is then recommended for distribution and commercial production. This does not mean that the final goal in wheat improvement has been reached. Wheat breeding

is a gradual process in which improvement in the various characters is made step by step. As the breeding program advances and further information is obtained on the nature and inheritance of characters, better varieties will continually be released that will possess high yielding ability, more winter-hardiness, greater disease resistance, and at the same time the desirable quality characteristic of soft winter wheats.

## SOFT WHEAT MILLING

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(Read at the Annual Meeting, May 1938)

In the mind of the writer there cannot be too much emphasis laid on the cleaning and conditioning of the wheat prior to milling. The cleaning, blending, conditioning, and the milling of the soft varieties of wheat deserve the same careful consideration as do the hard varieties. In fact, in the soft wheats in some sections of our country, we are confronted with a problem that is not generally experienced by millers of the hard varieties—that is, wheat that is infested with garlic or onions. This is more generally found in the regions south of the Ohio river and really gives the millers, in what we sometimes term as the Deep South, that is, the Carolinas, Virginia, Tennessee, and others, plenty to worry about. Fortunately, the soft-wheat millers further north are bothered very little if any.

Like most varieties of wheat grown, soft wheat has a certain amount of seeds, cockle, cheat, oats, and so forth. By using modern grain-cleaning equipment, the operator should have very little difficulty in eliminating this trash material. For instance, there has been a considerable improvement in the machines to eliminate seeds, cockle, oats, and so on. These machines are of the indented cylinder and disc type. Either one will do a very efficient job.

Where aspiration is required, you will find that most companies building grain-cleaning machines have greatly improved their equipment, especially where an air separation is used. In fact it is now possible for an operator to secure an aspirating machine that is self-contained. The use of such a machine in certain localities and plants has its advantages, one being that no air is expelled to the outdoors, which of course is a factor in cold weather. On the other hand, this type of machine is not as efficient as the disc-type aspirator, one where the air is blown into a dust collector of some type. These disc machines make a much better separation on fine dust particles.

As for the scouring equipment there have been many marked improvements during the past few years. We are now in a position to buy equipment that will do a very mild job and at the same time a moderate amount of aspirating. Then again we are able to purchase machines that have unusually well designed aspirating chambers, and at the same time they are in a position to offer partial carborundum cases when wanted and by so doing afford the operator a means of doing a very severe job of scouring the wheat when so required. In short, if one really wants to do a thorough job of grain cleaning today, the proper equipment is now available, and it is simply a matter of planning the installation carefully and then making the necessary purchases.

The problem of removing garlic or onions from wheat is a very difficult one and there are very few ways in which this can be done with any degree of success. The one method is that of drying the wheat down to a very low moisture content, which has a tendency to leave the garlic lighter than the wheat, which in turn makes it possible to remove by means of suction a considerable amount of the garlic from the entire stream. This system is a very costly method and is not extensively used for that reason. Another method that is recommended by one of the leading mill builders is as follows:

"We believe that the use of water on rolls is probably the most common method. In some cases the rolls are washed after shutting down, but where water is applied while the mill is in operation, the water is put on a very small section of the roll, which allows a dough ball to form. As this dries out, the centrifugal force of the roll throws off big chunks or sections. This is repeated down through the length of the roll, and this cleans the corrugations very readily. It is desirable with this system to put a scalping shoe below each pair of rolls while water is applied to remove the dough balls or chunks, thus preventing them from going down into the system and causing choke-ups in the sifters."

Where an operator is not equipped to remove garlic or use water as just mentioned, then he is left with only one alternative, and that is, he must shut down the plant whenever the roll corrugations fill up and give them a thorough scrubbing, because as might be expected, when these roll corrugations start to fill up, it is utterly impossible to reduce the wheat in a way that will conform with the general program of the mill. When the rolls are in this condition, it quite naturally affects the quality of the flour, percentages of the various grades, and also the yield.

After one has his wheat thoroughly cleaned he is then confronted



with the job of tempering or conditioning. By that I mean the amount of water necessary to put the wheat into proper condition for milling. In soft wheat raised east of the Rockies we seldom are confronted with the necessity of predampening. In my opinion, to secure the maximum of benefits from conditioning, the miller and the chemist should work hand in hand, because no matter how refined your milling process may be, without proper preparation of the grain one cannot hope to make the best product possible out of the grain furnished him for flour-making purposes. It is quite true that a great deal depends upon how the operator has his mill set or diagramed, the type of flour that it is necessary to make, etc. Grain conditioning is a most important part of the milling process and requires a great deal of study and a lot of attention.

The problems that one encounters in the breaking process differ greatly from year to year, and quite frequently after a crop starts to move the operator finds it necessary to alter the type of corrugations used. This sometimes does not have to be done all the way through the system. Frequently, by changing just a couple of the breaks, the results wanted can be secured.

As for the breaking of soft wheat and hard wheat on the same rolls, this presents quite a different problem. I mean that the operator is forced to use the type of corrugations that will give him a satisfactory yield and obtain the maximum results on the softer varieties of wheat. It should be quite apparent that if a very vitreous type of wheat is ground on these same machines, one cannot expect to secure good results for the reason that there is a tendency to shatter these hard berries, which makes it almost impossible to grade and purify the middlings in a way that is comparable to a mill designed for the milling of hard varieties only.

In designing a mill the engineer must constantly keep in mind a balance between the grinding capacity, the bolting capacity, purifying, and other factors. A mill is really made up of five distinct divisions: the breaking process; the sizings, or that part of the system where the large middlings are lightly ground so as to make it possible for one to eliminate the germ from the better stocks and purify a portion of the balance; the middlings system, which is that part of the system handling the choice flour-making parts of the berry; the tailings system, which is that part of the mill where one gathers up the fine brany particles, reducing them in a way so there is no loss of flour into feed; and the last is the low-grade or finishing systems where the last bit of good flour is obtained. It is, therefore, quite important that great care be given to the placing of the equipment for sifting out the flour and making the other separations,

All too often does the operator find that in the milling of soft wheat he is in no position to add to the bolting capacity. The reason he might want to add to this capacity is that occasionally the company desires to change the type of flour it is producing, or one may meet with a very soft crop and consequently find that it is imperative to do something to properly dust the flour out. If this cannot be done, the operator has only one alternative and that is to reduce the capacity of the mill to a point where the bolting machines will perform the work in a satisfactory manner.

Fortunately, during the past few years, there has been brought out by the various manufacturers of milling machines, a number of different types of small sifters. These run at a high speed with a small throw, and they really do have considerable capacity. When one finds himself short on sifting capacity and crowded for space, he may be able to use one of these small sifters to a great advantage. In fact such a machine inserted in the flow plan would have a tendency to make the mill quite flexible at any time.

Frequently the question has been asked of me, "Why is it that soft-wheat mills are so often bothered with choke-ups?" To me there really is very little excuse in the present-day flour mill for being bothered to any great extent with chokes, because it is simply a matter of studying the degree of pitch required in order that the various types of stock in a flour mill will flow freely. If one finds that the proper fall cannot be had because of the distance between floors or machines, he would be much better off installing a few short conveyors than be bothered with flour stocks spilling all over the floors and becoming contaminated with dirt.

## SOFT-WHEAT TESTING PROBLEMS

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(Read at the Annual Meeting, May 1938)

The literature of cereal chemistry dealt almost entirely with hard-wheat problems until a few years ago. The cereal laboratory had its origin and early development in the hard-wheat sections, and the millers of soft-wheat flours were not greatly interested in laboratory tests until they felt the pressure of loss of sales outlets. In earlier years the soft winter flours were commonly used in commercial bread production as well as for cakes, pastries, and crackers; but with improved milling and maturing technique, and the mechanized bread-making methods,

the hard-wheat flours largely displaced the soft with this most important class of consumers. It was at this point that the soft-wheat millers enlisted the aid of cereal chemistry to develop new outlets for their products; and specialized flours increased in numbers. The greater part of the output of the larger soft-wheat mills now is shipped to a variety of food manufacturers, each with his special performance requirements.

Hard-wheat flours are used almost entirely for making yeast-leavened bread and can be tested and judged rather uniformly according to certain standards for gluten strength and character, and enzymatic properties. The many uses to which soft-wheat flours are put requires that more of the flour characteristics must be controlled; and the soft-wheat laboratory must be prepared to conduct a wider variety of tests, particularly baking tests, than the hard wheat laboratory. Likewise the interpretation of soft-wheat tests requires a somewhat different viewpoint than in the case of hard-wheat tests. The utility and appreciation of soft-wheat flours is tied up more with delicacy, tenderness, flavor, and eating quality, rather than with strength, gassing power, and the ability to withstand "punishment" as in hard wheats. The term "strength" is also heard in connection with soft-wheat flours, but is used merely in a comparative sense. In very few cases is a "strong" soft-wheat flour comparable in gluten strength with even a "weak" hard-wheat flour. Another important difference is that hard-wheat flours are made with the expectation that they will be used in a manner which will greatly modify the gluten and other characteristics, with special reference to the action of dough mixers, yeast foods, and fermentation. Soft-wheat flours, on the other hand, are usually so balanced that they are ready to give the desired results with only the mild changes caused by a gentle mixing action.

The reason for specialized soft-wheat flours is that the best quality of products and greatest economy are realized through their use, in that they are milled and matured from selected wheats and mill streams to suit the bakers' processes.

The biscuit and cracker bakers require three types of soft-wheat flour, these being the cracker sponge flour, the cracker doughing and hard sweets flour, and the cookie flour. These differ considerably in both analysis and in performance and may have to be drawn from widely separated milling sections.

The larger cake bakeries usually employ three or four types of soft-wheat flour. There is the very short patent, highly bleached and finely granular flour, suited for angel foods and for formulas with a very high percentage of sugar and liquids and for cakes requiring a very white crumb. The next grade may be the longer patent, also well

bleached, and used for loaf cakes, the cheaper layers, sponge cakes, and general purposes. A third grade of flour may be used for pastries and a fourth grade for cookies.

Pretzel bakers and bakers of many other specialties have each their own requirements in soft-wheat flours. These requirements may be vastly different but have been found from experience to be necessary for the proper balance of quality and production costs in the plant in question. The point to be made here is that it has been found more profitable to assume the greater trouble and cost of obtaining specialized flours than to make the easily available flour work by changing formulas and processes. Smaller plants may be forced to this expedient by lack of capital or storage space, but larger plants carry the necessary line of flours.

It is unnecessary to remark that the production of specialized soft-wheat flours requires very close co-operation between wheat buyer, miller, and chemist, with the latter as the control man and source of information. The character of a flour is determined principally by the variety and quality of the wheat composing the milling mixture. The miller, in conjunction with the laboratory, may do much to bring out the proper qualities in a flour, but the basic character of the finished product must be founded on intelligent wheat selection and blending. Numerous soft-wheat varieties are being grown in the soft-wheat areas, and there are important differences in the baking properties of their flours. It has also seemed to the writer that, possibly because of a more irregular climate and rainfall in the soft-wheat sections, the soft wheats tend to show greater changes in character from one crop to another than is the case with hard wheats. These seasonal changes are made the more important because specialized soft-wheat flours must be more accurately balanced than bread flours need to be.

The narrow margins on which flour is sold prevent shipment of wheat for long distances except in seasons of extreme quality changes. This sometimes leads to pressure on mill production men, including chemists, to make them adapt available wheats, which would not be acceptable under more normal crop conditions. Thus we have noted attempts to offset extremely low gluten strength by blending strong hard wheat with the soft, or the blending of starch with the flour from wheats that were too high in gluten strength or had harsh characteristics. In these instances the analyses may be brought into line but not the baking qualities. In years of soft-wheat scarcity the cracker bakers have tried to use hard-wheat sponge flours, but to the best of my knowledge this penalized the appearance and eating qualities of the crackers too much to suit them. The same has been the case where high "strength" has been offset by using a diluent of starch in cake flours. Possibly such expedients may

be workable someday, but at the present time it is well to be wary of makeshifts in the production of specialized soft-wheat flours.

In addition to his wheat charts the soft-wheat mill chemist should have a detailed knowledge of the mill streams, the effects of bleaching and maturing agents, and the performance of his flours under varying baking and processing treatments. It may be necessary to work out a new type of flour on short notice, and this information should be at hand. An understanding of the action of doughs and batters is essential in selecting suitable flour for any purpose. It may be that under certain conditions a lower-grade flour is indicated rather than a short patent, or an unbleached rather than a bleached flour. Type of maturing agent is quite important, as is the flour granulation size. Green or under-aged flours may work best sometimes, or the use may indicate a flour which has been well aged and matured. The effect of treatment in flour driers is important, as marked alterations in colloids can take place in these machines under certain conditions. None of us will ever find out all we should know about our mills or our wheats, and spare time should be spent in a continual search for information and new methods for attacking our problems.

Commonly used tests in soft-wheat laboratories are those for percentage of moisture, ash, and protein, for absorptive power, color, viscosity, hydrogen-ion concentration, and granulation (A.A.C.C., 1935). There are a number of methods for determining gluten qualities, and the number of baking tests equals the number of uses for soft-wheat flours. Technical organizations, including the A.A.C.C., have had active committees working on these testing methods for years, and improvements are suggested almost annually. There is much friendly controversy respecting the merits of different methods of testing, which is a healthy, constructive condition. In the following discussion it is hoped that the opinions of the writer are accepted for what they are, that is, the results of experience, or the observation and application of information generally known and published.

The moisture test is empirical in character, and is reliable only when conducted under strictly controlled conditions. There are a number of devices for determining moisture in cereal products, and the physical make-up of the material tested has a great effect upon the type of apparatus. Water is understood to be present in flours in three forms—free moisture, combined moisture, and moisture of composition, and they are expelled from the tested flour in that order when the flour is exposed to heat and vacuum. The free moisture is expelled rather easily, but the moisture of composition passes off only when heating is sufficiently severe to scorch or partially break down the flour. Moisture tests should be made only by recognized, official methods, and the rules

for the test should be uniformly observed in order to achieve uniform results between laboratories. Flours and wheats tend to reach a state of moisture equilibrium with the surrounding atmosphere, and will take up and give off moisture according to whether the air is humid or dry. Hence laboratory samples should be taken and kept, until tested, in sealed containers of metal or glass.

Moisture is more closely held by some wheats and flours than by others. This was very apparent when we replaced the familiar Brown-Duvel moisture apparatus with the 130°C. drying oven in wheat temper control work. It was found that hard wheats, of higher protein content, checked in moisture results as between the two types of tests, but the soft wheats yielded about 1% higher moisture results in the severe drying oven as compared with the comparatively mild Brown-Duvel. The logical explanation was that the more severe method of drying released a greater amount of the combined moisture in the soft wheats, while the hard wheats withstood the same condition. In other words, the increased vapor pressure produced in the hard wheat by the oven method did not overcome the affinity of the hard wheats for moisture but did so in the soft wheats to some extent. We found, however, that by applying this 1% correction we were able to make this change in testing methods without disturbing our control schedule for tempering.

Soft wheats are grown in areas having milder climates and good rainfall, and usually come to market containing a larger percentage of moisture than in the case of hard wheats. At the same time soft wheats cannot safely be stored at as high a moisture content as hard wheats, and are more likely to sprout and spoil. Being of softer texture, soft wheats are not tempered as heavily or as long as hard wheats, and are milled with a lower moisture content at the first break roll. It is sometimes the case, in years of heavy rainfall at harvest time, that the wheat is received with too high a moisture content for milling and actually has to be dried before sending to the rolls.

One of the principal reasons for control of moisture in soft-wheat flours is to maintain soundness. This applies principally to such flours as go into the warm, sultry, Southern sections. Flours for this trade are, as a rule, heavily bleached, which in itself shortens keeping time, and in storage conditions where drying out is slowed and temperature is favorable for the growth of mold and other organisms, flour moistures should be held down at the mill. Flours milled at very high moistures seem to suffer a loss in gluten strength in milling, possibly because of the higher roll pressures and consequent heat under such conditions.

The ash test has been used by cereal chemists for many years, and there is a great deal of literature on the subject of ash in flour. Essentially this determination is made to help the production man in the mill

in the control of flour grades and uniformity, or to give the flour trade a guide for establishing grades and a basis for uniformity in flour shipments. Low ash content in a flour indicates that it is a short extraction, very cleanly milled, from wheat of low mineral content, or a little of all three. Most soft-wheat flours are required to have a soft, pliable gluten; and shorter-extraction flours, as indicated by low ash contents, usually have this type of gluten. As the ash content rises and the grade of flour goes down, the gluten will tend to be tougher and shorter. This is due, at least in part, to the buffering, binding action of the higher amounts of mineral matter in such lower grades. Higher ash content is also associated with darker flour color.

Soft-wheat flours tend to contain less ash, grade for grade, than hard-wheat flours; and the ash is more volatile when heated and more hygroscopic when cool. These properties indicate a difference in the mineral composition. Soft-wheat flours burn out more quickly than hard, and tend to fuse unless properly handled. We have had improved results in ashing soft-wheat flours since using the spun nickel crucibles recommended by H. W. Putnam in a paper which he read at the January, 1938, meeting of the Cincinnati section. With this type of crucible the tendency toward fusing of the ash or scaling of the crucible, as in the case of porcelain and silica crucibles, was greatly diminished.

Color scoring is one of the more important and probably one of the oldest means of grading flours. The only method which has been generally successful has been the Pekar or "slick" method, where the flours are smoothed down side by side on a paddle, then dipped in water and dried. There are objections to this method; and the method of handling, age, and moisture content of flour, and method of drying do affect the comparisons. Color analysis has been tried in which the proportions of red, yellow, black, and white have been determined, but these were not sufficient because they did not show the brightness or "bloom" which the trade associates with a properly milled flour.

While some special flours should be unbleached and of yellow color, the greater portion is required by popular demand to be white or bleached. The most satisfactory color results from the use of good wheat and proper milling, and the writer prefers to consider bleaching as a means of maturing the flour to bring out baking properties rather than merely a method of oxidizing the yellow pigment it contains. A properly matured flour is likely to show a better crumb color, the true measure of flour color, than one which has been bleached until the flour is very white. It is generally accepted that soft cookie or pastry flours should be left unbleached or else lightly treated with some agent that does not affect the gluten, such as  $\text{NO}_2$ . Soft flours to be used in bread or biscuits will be strengthened if treated with  $\text{NCl}_3$ . Cake flours

should be bleached with  $\text{Cl}_2$  and where an extra-white color is required, any of the above bleaching agents may be used in combination with benzoyl peroxide or a similar agent.

A test for the hydrogen-ion concentration is widely used to control the rate of chlorine bleaching of cake flours. Both electrometric and colorimetric or indicator methods are used. The electrometric or potentiometer method is more scientific and gives finer readings, but the use of the apparatus requires greater technical ability. Notable improvements in this type of equipment have increased its use in the past year or two. One of these improvements has been the perfection of a durable and accurate glass electrode; and at this time several makes of compact, self-contained pH meters are available, with standard cells for ready, quick reference in checking readings. However, many small laboratories continue to use colorimetric methods because they are adapted to the use of routine technicians with limited scientific training. This method is sufficiently accurate to control chlorine bleaching within satisfactory limits of variation.

Cake-flour millers have known for some years that a good chlorine bleach was essential for proper baking performance in light cakes. The action of the chlorine bleach has never been worked out in detail, but it has been observed that flours with too high a pH, if used in connection with rich formulas, tend to rise in the oven but fall or shrink when taken from the oven. Some light is shed on the subject by L. H. Thomas, an expert in the manufacture of wheat starch and gluten derivatives, and I quote him directly (personal communication, 1938).

Chlorine has been used for years to produce the so-called "thin boiling starches," which are starches which reduced capacity for swelling in boiling water. More recently it has been found possible to control this process; and it has been discovered that in the initial stages the swelling capacity of the chlorinated starch has been greatly increased over that of the natural starch.

With regard to the dispersion of gluten by negative ions, we have found it impossible to wash out gluten in our commercial starch process if the flour has been previously treated with chlorine. In the laboratory we have studied the dispersion of pure gluten, and found that at a pH between 5.2 and 3.0 the gluten was dispersed into such a colloidal condition that at certain concentrations it could be whipped much like egg whites.

The dispersion of the gluten by chlorination reduces the doughing tendency of the flour; and mixing with other ingredients of the cake batter undoubtedly keeps the gluten dispersed even though the pH of the batter is higher than that of the flour. Another factor may be of major importance. That is the greater capacity of the dispersed gluten for taking up water. Naturally any ingredient which takes up water in this way must have a material effect on the character of the cake batter.

Mr. Thomas has found that chlorination of flour produces marked dispersion of the gluten, as well as an increase in the hydration capacity of the starch and gluten in batters. The practical baker knows that he cannot raise the percentage of sugar in a cake formula without also increasing the liquids. The moisture-carrying capacity of a flour, as



well as the pliability of its gluten, determines its performance in a cake formula. Thus the effect of bleaching cake flours with chlorine is somewhat clarified. High-grade cake flours are usually chlorinated to a pH of between 5.0 and 5.2. The amount of chlorine necessary to do this will depend upon the extent to which the flour is buffered, but one ounce of chlorine per barrel will reduce the pH of a patent flour about 0.25 pH unit if the flour consists mainly of streams from the purified middlings stock. The degree of chlorination necessary for best baking results will vary a little from crop to crop.

Flours to be used in biscuit and bread baking should not be subjected to the gluten-dispersing action of chlorine, as they need all the gluten strength possible in such naturally weak flours. Flours treated with chlorine tend to make somewhat sticky doughs, which are not easy to handle and mold. A low pH is also undesirable in flours designed for cookies and pastries which are supposed to bake out tender and crisp. Chlorinated cookie flours will be reduced in plasticity of dough, or spreading ability, and will lack spread, top grain, and crispness unless the formula is enriched or additional leavening is added.

One of the first papers published in *Cereal Chemistry* on the subject of soft-wheat flour was by Patterson (1924), and dealt with the importance of flour particle size in cake flours. He recommended as fine a particle size as was economically feasible. Other work done since then has emphasized that a measure of control should be exercised over cake-flour granulation. It seems likely that the significance of granulation in this case is tied up with the quantity of damaged starch granules present in the flour. The number of these would, of course, be greater in a flour reduced to pass through a bolting cloth with very fine apertures. Alsberg and Griffing (1925) have shown that when the envelopes of starch granules are bruised or broken they take up cold water as fast and to as great an extent as do boiled, undamaged granules. In cake making a foamy structure is built up out of eggs, sugar, and shortening, and then the flour is incorporated with the minimum of mixing to produce a smooth, even batter. A flour such as indicated above, which would combine quickly with the liquids, would require a minimum of mixing time and thus would avoid destruction of the foam structure by overmixing and would have less tendency toward additional water absorption on standing and consequent changing of the batter consistency.

In our laboratory we use a standard Rotap sifter for granulation work. We do not use metal sieves, but use the eight-inch sieve frames and regular mill bolting cloths. Our procedure on soft-wheat flours is as follows: We nest three sieves, 10 XX, 12 XX, and 14 XX, and place 100 grams of the flour on the top sieve, and Carmichael cloth cleaners on each sieve. The sifter is run five minutes, then stopped. The sieves

are carefully dissembled and tapped to remove flour from around the edges and from the tops of the cleaners, with care taken not to lose any of the flour. The sieves are re-assembled and returned to the sifter, which is run for an additional ten minutes. The portions on each sieve and through the 14 XX are then weighed and calculated to percentages. It is our experience that results can be checked within about 2% after the technique is developed. The flour moisture content is a factor, of course, but not as great as would be expected. The interrupted sifting operation was found necessary because of the sticky nature of most soft-wheat flours. It would not be as necessary on more granular flours. We recommend round sieves for this work because they have no corners for the flour to lodge in, and good cloth cleaners are also quite important.

We have heard the comment that this sifting test differs from the bolting action of the commercial flour mill, but what we want is a comparative test for granulation size, and this method has proved serviceable. It lends itself to the control of mill-stream granulation, finding leaks in mill machines, controlling uniformity of bolting, and the comparison of finished flours. Most of the better cake flours will bolt 98% or more through the 14 XX cloth by the method mentioned. We do not use a cloth finer than 14 XX because baking tests do not show that it is necessary. However, when the flour granulation was much coarser than this "98% through the 14 XX," there were definite indications of lowered cake-making quality. We have done little work concerning the significance of granulation in bread and biscuit flours, but we believe that the more granular type of soft-wheat flours, containing fewer damaged starch cells and capable of withstanding more punishment in mixing, would be preferable.

In soft-wheat flours, just as in the hard-wheat, protein quantity and quality, absorption capacity, the various phases of gluten character, and the machines for testing them are to be considered in one group.

A physical test which has been utilized, and which has been under discussion for years, is the viscosity test. Many factors may influence viscosity readings, such as the mineral content of the flour, nature and rate of bleaching, and quantity and quality of gluten, but in general the higher readings indicate greater hydration capacity, greater gluten "strength," and higher grade of flour. In addition to furnishing a picture of the gluten character, the A.A.C.C. viscosity test also permits the plotting of a curve based on viscosity increases as against increments of added lactic acid. The shape of this curve will indicate the manner in which the flour is buffered, and hence the probable grade. One type of viscosity test eliminates the effect of quantity of protein by using an amount of flour in each case equivalent to exactly two grams of protein. Bayfield (1936) has shown that the variations in the bulk of flour used

in this test had but little effect on the viscosity readings. The most generally used type of test, and the one discussed in connection with the addition of increments of acid, uses 20 grams of the flour (15% moisture basis), and does not consider varying percentages of protein.

The biscuit and cracker industries place a high value on the viscosity test, and most such concerns buy flour under rigid specifications for viscosity. Cake flours are not usually purchased under viscosity requirements, but they might well be, with an advantage to the purchaser. Tests conducted with the MacMichael viscosimeter, a torsion-type instrument, are usually specified for flour measurements. In addition to following the grade of flour, viscosity readings appear to be closely connected to wheat variety and growing section. In the Michigan-Ohio district the readings on similar grades of flour will run approximately half as high on the soft white winters as on the soft red winters. This differential may be made quite useful when viscosity specifications fall somewhere between the average viscosities of the two types, and the wheat mixture can be used very nicely to control the viscosity.

Recording dough mixers such as the Brabender farinograph and the Swanson recording mixer can also be adapted for testing soft-wheat doughs. Unless the mixer speeds are slowed down, however, the soft-wheat dough will break down too rapidly to allow formation of a readable curve. In our laboratory we have a Brabender farinograph with a slow-speed mixer for soft-wheat flours and a high-speed mixer for hard-wheat flours. This apparatus has been found useful for determining the speed of dough development, capacity for taking punishment, comparative dough elasticity, and water absorption, and also for recording a permanent record of these determinations. More care is required in a mixer test of a soft-wheat than of a hard-wheat flour on the farinograph. Usually the dough will start to break soon after it has formed, and absorption determinations by the addition of increments of water are difficult.

The specialized soft-wheat flours, which are usually required to be nicely balanced, depend quite a bit on the gluten quantity-quality ratio, with the greater emphasis on gluten quality. Angel-food cake flours seem to do best with a small percentage of firm, elastic gluten. Other cakes require flour with a larger percentage of soft, spongy gluten. Cookies require a flour with a small quantity of somewhat short gluten. Cracker sponge and pretzel flours should run high in quantity and strength of gluten, and so should most flours to be used for hot breads and biscuits and for yeast breads. Flour gluten is probably the most important single factor in the determination of soft-wheat flour character.

Soft-wheat laboratory tests should be confirmed, if possible, by a

scientific baking method adapted to the flour under consideration. The official A.A.C.C. bread-baking test (pup loaf) is being used with excellent results to check and classify flours used in the biscuit and cracker bakeries. This test has also been used to test cake and pastry flours, but in the judgment of the writer it does not give the proper picture here, and is likely to be misleading. Cracker sponge and dough flours are fermented like bread doughs, but cake flours are handled entirely differently, and cake flours must meet conditions not checked by a bread-baking test.

A standard cake-baking test is difficult to develop because there are a number of distinct types of cake-making procedures, all differing in formula and mixing methods. However, an experienced operator can get a good idea of the general possibilities of a cake flour by judging its performance in a layer and loaf formula. Sponge-cake tests seem better suited to test flours for sponge and angel-food cakes because a very small amount of flour is used in an angel-food batter in connection with a large percentage of egg whites, and the quality of the egg whites and the way they are beaten is a more important consideration than a small variation in flour quality. In recent years the sugar and shortener tolerance of cake flours has been increasingly important with the use of richer commercial formulas. Shortener tolerance is allied with gluten strength, with the greater gluten strength requiring increased amounts of shortening. The manner in which sugar tolerance is tied up with granulation, hydrogen-ion concentration, and absorption has already been discussed.

A simplified commercial cookie test was suggested by the writer (Alexander, 1933) and has been used, with few modifications, since then. This test is of value for testing cookie and pie flours and other flours to be used in products which are prepared from doughs in which the gluten is not too fully developed. In this test the symmetry of the cookie, the spread, top grain, internal structure, and thickness are scored and recorded; and a factor based on cookie diameter divided by cookie thickness is used.

The biscuit-baking test is used to evaluate most of the plain, phosphated, or self-rising flours going into the southeastern states for use in the hot breads favored there. This test is the result of years of committee work in the A.A.C.C. In the opinion of the writer, all of the baking tests developed by the A.A.C.C. committees are quite serviceable, although some of them are still tentative tests and will be further improved before being made official.

Proper and uniform scoring is a very important part of all test bakes and this comes only from experience. It is agreed that there are good possibilities of improving our scoring charts so as to make them

more suitable for the use of operators lacking in experience. There are two viewpoints from the standpoint of scoring test bakes. The mill chemist is inclined to look backward through the milling and bleaching processes toward the wheat mixture and the bakery chemist looks forward through the bakery formula and mixing procedure toward the finished bread, but the same kind of baking test should suffice for both.

In this rather lengthy paper we have touched on many of the problems and methods of the flour-mill chemist, but by no means all of them. Those in other lines of work, who still think of flour milling as in the grist mill era, often ask, "What does a chemist find to do around a flour mill?" The correct answer to this query is, "Plenty."

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## WHEAT IMPROVEMENT IN THE EASTERN UNITED STATES

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The wheat-improvement program in the eastern United States is of direct interest to the cereal chemist because of its effect on quality of the grain marketed. If the plant breeders and others interested in the wheat-improvement program carefully analyze all the needs of the trade, it should be possible to raise the quality of grain produced as well as reduce the losses to farmers caused by winterkilling, diseases, insects, and other hazards. One of the causes of variability in wheat marketed is the large number of varieties now grown. There are good reasons to believe that the number of varieties in the soft-wheat region in the eastern United States could be limited to a dozen without reducing the total production in this region. This certainly would be the case if the plant breeders were to add, as can be done by more extensive use of backcrossing, more winterhardiness and resistance or tolerance to leaf rust, stem rust, loose smut, stinking smut, Hessian fly, etc., to

selected varieties of soft red winter wheat. The purpose of this discussion is to give some idea of the distribution of varieties in this region, of losses due to specific crop hazards, and the possibility of reducing these losses through plant breeding.

### Distribution of Classes and Varieties

Over a period of years, farmers will learn by experience which varieties give the best returns in their locality. If we consider the varieties which have been available for several years, a study of their distribution should indicate rather accurately the types that are best adapted. Varieties may be grown by a few farmers after they have been discarded by most, but a variety or type that is grown on a high proportion of the acreage over a wide area must be well adapted. It is these widely adapted types that should be used as a basis for the wheat improvement program. The latest distribution data available are based on the 1934 crop. Figure 1 shows the approximate distribution of the

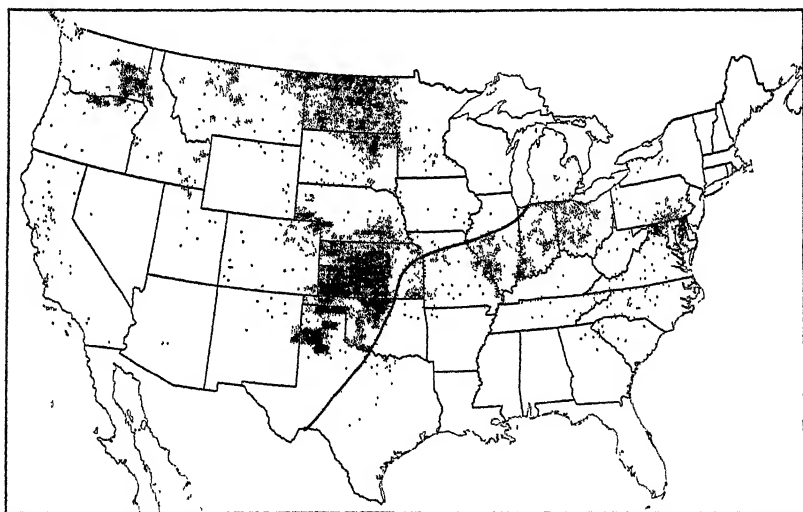


Fig. 1. Distribution of all wheat in the United States in 1934. Soft wheat is the principal kind grown east of the heavy black line. Each dot represents 5,000 acres.

total wheat acreage in the United States in 1934.<sup>1</sup> Soft red varieties were grown on about 12,750,000 acres and are the predominating class grown east of the heavy line on the map. They constituted practically all of the wheat grown in the region extending as far west as eastern Kansas, with the exception of some hard red winter wheat in central Illinois and some soft white winter wheat in western New York and in Michigan. Twenty-one varieties of soft red winter wheat were grown

<sup>1</sup> For more complete information on the distribution of varieties the reader is referred to U. S. Dept. of Agr. Circ. 424.

on more than 150,000 acres each and 52 varieties on smaller acreages, many of them on only a few hundred acres. Soft white winter varieties were grown on about 710,000 acres in the eastern states. Only two varieties of this class were grown on more than 150,000 acres each, and eight additional varieties on smaller acreages. The estimated acreages for those soft red winter and white winter varieties grown on more than 150,000 acres each are given in Table I. If the acreages of very

TABLE I

VARIETIES OF SOFT RED WINTER AND WHITE WHEATS GROWN IN THE EASTERN UNITED STATES ON MORE THAN 150,000 ACRES EACH IN 1934<sup>1</sup>

Type or variety	Acreage of	
	Variety	Type or variety
	1,000 acres	1,000 acres
RED GRAIN		
<i>Fultz type</i>	—	3,544
Fultz	1,870	—
Trumbull	1,136	—
Fulhio	534	—
Ashland	4	—
<i>Fulcaster type</i>	—	1,910
Fulcaster	1,395	—
Nittany	409	—
V.P.I. 131	106	—
Red May	—	977
<i>Mediterranean type</i>	—	788
Mediterranean	519	—
Red Rock	220	—
Denton	49	—
Leap	—	709
<i>Poole type</i>	—	686
Poole	673	—
V.P.I. 112	13	—
<i>Purplestraw type</i>	—	597
Purplestraw	306	—
Flint	178	—
Redhart	112	—
Gasta	1	—
Currell	—	480
Harvest Queen	—	380
Red Wave	—	307
Purkof	—	300
Forward	—	258
Rudy	—	212
Nigger	—	152
WHITE GRAIN		
<i>Dawson type</i>	—	425
Dawson	356	—
Honor	69	—
Goldcoin	—	243
Total		11,968

<sup>1</sup> Fifty-two other varieties of soft red winter and 8 varieties of soft white winter wheat were grown on less than 150,000 acres each or a total of about 782,000 acres.

similar varieties such as Fultz and the closely related varieties Trumbull, Fulhio, and Ashland that were selected from Fultz are combined, it is found that the Fultz type was grown on about 3,500,000 acres in 1934, concentrated in the area just north of the Ohio river.

The Fulcaster type, including Nittany and V.P.I. 131, was grown on nearly two million acres. This acreage extends throughout the Piedmont section from Pennsylvania, Delaware, and Maryland to Georgia. Rather concentrated areas are also found in central Tennessee, southeastern Kansas, western Missouri, and central Oklahoma.

Red May, commonly known as Michigan Amber in Indiana and Michigan Wonder in Missouri, was grown on nearly a million acres, mostly in Indiana and Missouri. The Mediterranean type, including Red Rock and Denton, was grown on about 750,000 acres. Red Rock is limited chiefly to Michigan and Denton to Texas.

Leap was grown on about 700,000 acres, mostly in the Piedmont from Pennsylvania and New Jersey to North Carolina. The Poole type was grown on about 700,000 acres, mostly in Ohio, Indiana, Kentucky, and Missouri. The Purplestraw type was grown on about 600,000 acres in Virginia, the Carolinas, Georgia, and Tennessee. Currell was grown on about 480,000 acres and Harvest Queen on 380,000 acres, mostly in eastern Kansas, Oklahoma, and Missouri.

About 300,000 acres of Red Wave were grown over a wide area. The 300,000 acres of Purkof were mostly in Indiana and Illinois. The 250,000 acres of Forward were scattered from New York to North Carolina, with the only concentrated area in southeastern Pennsylvania. The 200,000 acres of Rudy were mostly in Indiana. The 150,000 acres of Nigger were in Ohio and Indiana with a few thousand acres in southeastern Kansas.

Only two white wheats are of importance in the eastern states. The Dawson type, including Dawson and Honor, was grown on 425,000 acres, mostly in Michigan and New York; and Goldcoin on 243,000 acres, mostly in New York, northern Ohio, and Michigan.

Many of the 52 varieties of soft red winter and 8 of soft white winter grown on less than 150,000 acres each, or a total of about 782,000 acres, could profitably be replaced by better varieties.

### Causes of Loss and Their Control

Farmers are going to grow the varieties that they believe will give them the greatest profit. This profit is determined chiefly by the acre yield and the price received for the grain. They are not concerned with quality except as it is reflected in the price. Acre yield is determined by a number of factors such as soil fertility and moisture, as well as by characteristics of the plant itself. The present discussion



is concerned only with the plant characteristics that determine the relative yields and quality of varieties in the wheat-growing areas. An attempt will be made to give some idea of (1) the important factors that cause heavy losses and the areas where these losses occur, (2) differences in varieties with respect to the particular characteristics, and (3) the progress that can be expected in improving standard varieties with respect to some of these characteristics.

### Winterkilling

The losses caused by winterkilling in the soft red winter region are probably greater than the combined losses from all plant diseases. The acreage abandoned in the year 1928 when very heavy killing occurred and the average percentage of the acreage abandoned for the years 1909 to 1937 are shown by states in Table II. While no reliable

TABLE II  
PERCENTAGE OF ACREAGE OF WINTER WHEAT ABANDONED BEFORE HARVEST<sup>1</sup>

State	Acreage abandoned	
	1928	1909-1937
	%	%
Illinois	62.0	10.9
Indiana	60.0	9.3
Ohio	64.0	9.1
Kentucky	65.0	9.2
Missouri	32.0	7.9
Arkansas	30.4	8.6
Tennessee	28.1	6.2
Georgia	14.5	7.9
South Carolina	11.5	5.2
North Carolina	7.0	3.0
West Virginia	15.1	3.3
Virginia	6.0	2.4
Maryland	2.9	2.6
Delaware	0.9	2.9
New Jersey	5.2	3.8
Pennsylvania	9.0	2.9
Michigan	10.0	4.7
New York	6.1	3.7

<sup>1</sup> Data for 1909-34 compiled from "Revised Estimates of Wheat Acreage, Yield, and Production 1866-1934," September, 1934, reissued May, 1937, mimeographed report of the Bureau of Agricultural Economics; data for 1935-37 compiled from unpublished records of the Bureau of Agricultural Economics.

estimates of acreage abandonment assignable to specific causes are available, it is believed that most of that recorded in Table II was due to winterkilling. Average abandonment in Illinois, Indiana, Ohio, and

Kentucky has been about 10%. Over 60% of the acreage of each of these four states, which grow about half of the soft red wheat crop, was abandoned following the severe killing in 1928. Missouri, Arkansas, Georgia, and Tennessee have a slightly lower abandonment, averaging about 7.5%. The average abandonment in the area east of the Allegheny Mountains is less than 5% in every state except South Carolina, where it is 5.2%. It is also interesting to note the abandonment of only 4.7% in Michigan and 3.7% in New York. Apparently the greater protection of the snow cover and more continuous low temperatures during the winter account for the better survival in these two states, as the varieties are known to be less hardy than those grown in Ohio, Indiana, and Illinois.

It is a well-known fact that wheat varieties differ greatly in their resistance to winterkilling. It is also well known that the plants may be killed either by low temperature or heaving, or a combination of the two, and by other environmental conditions. In the Great Plains drought also causes heavy abandonment, but in the soft red winter region this seldom is a factor and the difference between the seeded and harvested acreages is caused primarily by winterkilling from low temperature and heaving. Results from an extensive series of experimental tests during the years from 1933 to 1937 indicate that low temperature may have been the more important cause of killing during this period, although heaving is considered to have been the chief cause of killing in the soft red winter region previous to this time. In these tests, 30 varieties have been grown under comparable conditions at about 25 locations in the eastern soft wheat area in each of the last five years, making a total of about 125 tests. In 34 of them, partial killing of medium-hardy varieties was attributed mainly to low temperature. The reports indicate that in only four tests has killing been caused by heaving. Little or no killing has occurred in 87 of the tests. It may be that these tests have not been continued long enough or for other reasons do not properly represent the conditions in this area, and it is also likely that heaving, although not apparent, has contributed to injury by low temperature.

A summary of the results from those tests in which differential killing occurred is presented in Table III. In the tests where low temperature was the chief cause of killing, Minhardi, Minturki, Wisconsin No. 2, and Illinois No. 2 had average survivals of over 80%, whereas Leap and Purplestraw averaged only 25.5% and 32.4% survival. The varieties Red May, Fulhio, Trumbull, Poole, and Rudy, which are commonly grown in Ohio, Indiana, and Illinois, averaged only 70.3, 65.1, 64.9, 60.1, and 57.7% respectively.

Purkof, which averaged 79.8% survival, probably owes much of its

TABLE III

SURVIVAL OF VARIETIES IN THE EASTERN NURSERIES WHERE DIFFERENTIAL KILLING WAS CAUSED CHIEFLY BY LOW TEMPERATURE AND BY HEAVING, 1933 TO 1937<sup>1</sup>

Variety and C.I. No. <sup>2</sup>	Low temperature		Heaving	
	Av. 34 station years	Rank	Av. 4 station years	Rank
	%		%	
Minhardi (5149)	85.6	1	46.4	27
Minturki (6155)	82.4	2	49.9	26
Wisconsin Pedigree No. 2 (6683)	80.6	3	—	—
Illinois No. 2 (11537)	80.6	3	72.0	12
Purkof (8381)	79.8	5	60.1	24
Kawvale (8180)	77.6	6	73.9	8
Kharkof (1442)	75.9	7	45.8	28
Harvest Queen (6199)	72.1	8	65.5	20
Red May (Michigan Amber) (5620)	70.3	9	70.8	16
Baldrock (11538)	69.3	10	86.1	1
Purdue No. 1 (11380)	67.6	11	83.4	2
Mediterranean selection (11567)	65.9	12	73.6	9
Fulhio (6999)	65.1	13	72.8	10
Trumbull (5657)	64.9	14	71.3	14
Fulcaster (6471)	63.5	15	78.5	5
Poole (3488)	60.1	16	71.0	15
Goldcoin (Junior No. 6) (6971)	57.9	17	56.8	25
Nabob (8869)	57.7	18	80.5	4
Rudy (5656)	57.7	18	83.0	3
Forward (6691)	57.4	20	70.8	16
Nittany (6962)	57.1	21	69.1	18
Gladden (5644)	56.5	22	74.6	6
Dawson (American Banner) (6943)	56.4	23	62.7	23
Valprize (11539)	55.7	24	64.3	22
Currell (3326)	55.5	25	72.1	11
Honor (6161)	54.4	26	68.5	19
Red Rock (6951)	53.1	27	74.5	7
Purplestraw (1915)	32.4	28	71.7	13
Leap (6958)	25.5	29	64.6	21
Redhart No. 2 (11654)	5.1 <sup>3</sup>	—	—	—

<sup>1</sup> Uniform nurseries maintained by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, in cooperation with the State agricultural experiment stations in the soft red winter wheat region.

<sup>2</sup> Accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

<sup>3</sup> Average of 31 station years. Leap for same years was 23.0.

productiveness and popularity to its hardiness. It is interesting to note that Goldcoin (Junior No. 6), Forward, Dawson (American Banner), Valprize, Honor, and Red Rock, the chief varieties grown in Michigan and New York, all average less than 60% survival, yet as shown in Table II the average killing in these two states is less than in states to the south.

Diseases such as leaf and stem rust, mildew, and leaf and glume blotch also are very destructive in lodged grain. The quality of grain from fields that are badly lodged is often injured by diseases, weathering, and sprouting.

Most of the commercial soft red winter varieties have fairly stiff straw but many are rather tall and are excelled in resistance to lodging by some of the newer varieties such as Valprize. It should be possible to introduce more resistance to lodging into the soft red varieties and thus reduce the losses due to lower yield and quality.

### Leaf and Stem Rust

The rusts have for centuries taken an intermittently heavy toll from the wheat crop. Estimates of the combined losses from leaf and stem rust in the states growing mostly soft red winter wheat are given in Table V for the years 1935 and 1937, when severe epidemics oc-

TABLE V  
ESTIMATED LOSSES FROM RUST IN BUSHELS AND IN PERCENTAGES IN THE STATES  
PRODUCING CHIEFLY SOFT RED WINTER WHEATS, 1909-37<sup>1</sup>

State	1935		1937		Average 1909-1937	
	1,000 bushels	Per-centage	1,000 bushels	Per-centage	1,000 bushels	Per-centage
Missouri	2,418	10.0	17,660	30.0	2,944	10.7
Illinois	1,567	5.0	9,613	16.0	1,844	5.4
Ohio	2,120	5.0	3,553	7.0	2,169	5.1
Indiana	320	1.0	9,920	22.0	651	1.7
Michigan	964	5.0	1,572	3.0	985	4.9
Virginia	271	3.0	270	2.5	271	3.0
Tennessee	0	0.0	780	10.0	27	0.3
North Carolina	0	0.0	485	7.0	17	0.2
Kentucky	0	0.0	549	5.0	19	0.2
Pennsylvania	0	0.0	4,622	12.3	159	0.4
Maryland	0	0.0	97	1.0	3	T
West Virginia	5	0.2	0	0.0	5	0.2
New York	0	0.0	2,340	20.5	81	0.7
Delaware	0	0.0	0	0.0	0	0.0
New Jersey	0	0.0	0	0.0	0	0.0
South Carolina	—	—	0	0.0	—	—
Georgia	—	—	638	30.0	—	—

<sup>1</sup> Data compiled from the records of the Plant Disease Survey, Mycology and Disease Survey, Bureau of Plant Industry.

curred, and the average for the period from 1909 to 1937. It will be noted that, while the average losses have not been very high, the losses in some years and in certain areas have been heavy.

The heaviest losses in the soft red winter region in most years are caused by leaf rust, but in 1937 the loss from stem rust was heavy in some states. Stem rust losses were estimated at 30% in Missouri,

12% in Indiana, 8% in Tennessee, 2% in Ohio, 2.5% in Michigan, and 2% in Virginia. The losses from leaf rust in 1937 were heaviest in New York, Pennsylvania, North Carolina, Illinois, and Ohio. Both rusts cause such heavy losses that breeding for resistance should be included in a well-rounded improvement program.

Breeding for resistance to stem and leaf rust has progressed much farther in the hard red spring wheats than in the winter wheats. Thatcher, which has been commercially grown since 1934, suffered very little loss in the severe stem rust epidemics of 1935 and 1937, and other new hard red spring hybrids not yet distributed are even more resistant to stem rust than is Thatcher, and are also resistant to leaf rust. Marked differences in resistance to leaf and stem rusts are found in the winter wheats. Results for some varieties grown in uniform rust nurseries at a number of locations in 1935, 1936, and 1937 are shown in Table VI. A number of new hybrids and selections are

TABLE VI  
REACTION OF WHEAT VARIETIES TO LEAF AND STEM RUST IN  
THE UNIFORM NURSERIES, 1935-37<sup>1</sup>  
(Average infection coefficients)

Variety or cross and C.I. No.	Leaf rust			Stem rust		
	1935, 18 nurs- eries	1936, 13 nurs- eries	1937, 10 nurs- eries	1935, 4 nurs- eries	1936, 6 nurs- eries	1937, 8 nurs- eries
Minhardi (5149)	81	55	57	42	55	59
Minturki (6155)	74	42	58	20	30	33
Mediterranean (3332)	59	38	39	76	56	72
Mediterranean selection (11587)	19	9	9	75	55	73
Kawvale x Tenmarq (11669)	—	14	12	—	27	29
Kawvale x Tenmarq (11750)	—	4	11	—	30	30
Hard Federation x Kawvale (11753)	—	2	6	—	31	51
Fulcaster x Tenmarq (11751)	—	2	5	—	31	40
Wabash (11384)	5	1	3	55	55	54
Hope x Hussar (11682)	1	1	1	1	2	T
Hussar (4843)	36	31	30	46	48	63
Mediterranean x Hope (11763)	—	—	4	—	—	4

<sup>1</sup> Data for 1935 and 1936 compiled from "Report of the Cooperative Uniform Cereal-Rust Observation Nurseries for the Year 1935," Jan. 15, 1937, and "Report of the Cooperative Uniform Cereal-Rust Observation Nurseries for the Year 1936," June 28, 1937, U. S. Dept. Agr., Bur. Plant Indus., Div. Cereal Crops and Diseases (Unnumb. Pub., Mimeographed). Data for 1937 compiled from notes taken by H. B. Humphrey, M. N. Levine, E. C. Stakman, C. O. Johnston, R. M. Caldwell, W. M. Bever, and H. C. Murphy.

highly resistant to leaf rust and somewhat resistant to stem rust. Those of most interest are Hope x Hussar (C.I. 11682) and Mediterranean x Hope (C.I. 11763). These two winter varieties received their resistance from the Hope parent and have been highly resistant to both leaf and stem rust. They are not suitable for commercial pro-

duction, however. The next step in the breeding program was to cross them with the commercial types of soft red wheat. From these crosses made at La Fayette, Ind., Manhattan, Kans., and College Station, Tex., lines are being selected that retain resistance to both leaf and stem rusts and that have grain and plant characters more nearly like the commercial parents. Wabash, which is highly resistant to leaf rust, is being recommended in Indiana and Illinois.

### Loose Smut

The average estimated reduction in yield caused by loose smut for the eastern states for the period from 1917 to 1936 is shown in Table VII. The heaviest losses occurred in the Piedmont and moun-

TABLE VII  
AVERAGE ESTIMATED REDUCTION IN YIELD CAUSED BY LOOSE SMUT IN THE  
EASTERN STATES, 1917 TO 1936<sup>1</sup>

State	Reduction in yield	State	Reduction in yield
	%		%
Virginia	2.6	Missouri	1.7
West Virginia	2.6	Indiana	1.7
Pennsylvania	2.4	Texas	1.5
Georgia	2.4	Oklahoma	1.4
Arkansas	2.4	New Jersey	1.4
Maryland	2.2	Illinois	1.3
Kentucky	2.1	Ohio	1.3
North Carolina	2.0	New York	1.2
Michigan	1.8	Tennessee	1.1
South Carolina	1.7	Delaware	0.7

<sup>1</sup> Data from records of Plant Disease Survey, Mycology and Disease Survey, Bureau of Plant Industry.

tainous areas in Virginia, West Virginia, Pennsylvania, Georgia, Maryland, North Carolina, and Kentucky, which have had estimated average losses of from 2 to 2.6%. Losses in the other soft red winter states are estimated at from 1 to 2%, with the exception of Delaware, where they have been lower. Losses have been estimated as high as 5% for some years in some states, and individual fields have been observed with more than 30% of the heads smutted. Breeding for resistance to loose smut has been given little consideration largely because a satisfactory method of testing for resistance has not been developed. Recently, however, methods of inoculating the flowers have been improved and more work is being done on this problem.

It has been shown that there are several races or varieties of loose smut and until more survey work has been done to check the races commonly present in the wheat-growing areas the value of resistant varieties cannot be predicted definitely. However, varieties are

available as parents for crossing which have not been infected with any collection of smut to which they have been subjected.

The reaction of the more resistant of a large number of varieties tested in the years from 1923 to 1928 is shown in Table VIII, together

TABLE VIII  
PERCENTAGE OF SMUTTED HEADS IN VARIETIES OF WINTER WHEAT  
WHEN HAND-INOCULATED WITH LOOSE SMUT<sup>1</sup>

Class and variety and C.I. No.	Years tested	Total heads	Smutted heads
	No.	No.	%
<i>Soft Red Winter:</i>			
Forward (6691)	3	946	0.0
Fulcaster (3605)	3	658	0.5
Leap (4823)	3	1,873	1.1
Purplestraw (1915)	3	867	0.9
Sol (6009)	2	1,398	0.0
Trumbull (5657)	2	449	0.0
Red Rock (5976)	2	928	24.0
<i>Hard Red Winter:</i>			
Blackhull (6251)	3	434	0.0
Hussar (4843)	2	1,764	0.4
Ridit (6703)	2	1,443	0.0
Turkey (1558)	1	775	46.4

<sup>1</sup> V. F. Tapke, Influence of varietal resistance, sap acidity, and certain environmental factors on the occurrence of loose smut in wheat, J. Agr. Research 39: 313-339, Sept. 1, 1929.

with the infection in two susceptible varieties. Several soft red winter varieties including Forward, Leap, Trumbull, and Purplestraw were highly resistant. To inoculum used in 1937 they were, however, susceptible. Kawvale and Illinois No. 2, not included in the earlier tests, were resistant. It seems likely that the inoculum used in the later experiments contained additional races. If these two varieties continue to be resistant to other races, it should be comparatively easy to get resistance into the commercial soft red winter varieties by hybridization. The hard red spring variety Hope has been inoculated with several races and has been very resistant in all tests.

### Bunt, or Stinking Smut

The percentages of soft red winter wheat receipts at the terminal markets in the years from 1928 to 1936 that were graded smutty are shown in Table IX. These data are compiled from the grain inspection records of the Grain Division of the Bureau of Agricultural Economics. By far the heaviest losses occurred in Maryland and Pennsylvania, where averages of 22.0% and 14.6%, respectively, of the wheat received at inspection points were graded smutty. In the important soft red winter states, Michigan, Ohio, and Indiana, 2% or more of

the receipts graded smutty, and in several other states more than 1% graded smutty. Of all soft red winter receipts, except at the Pacific Northwest terminals, 3.2% graded smutty.

TABLE IX

SUMMARY OF CARS OF SOFT RED WINTER WHEAT WHICH GRADED SMUTTY WHEN INSPECTED AT TERMINAL MARKETS IN THE EASTERN STATES IN THE NINE CROP YEARS, 1928-1936<sup>1</sup>

States	Cars inspected		
	Total	Graded smutty	
	No.	No.	%
Alabama	52	1	2.0
Illinois	27,253	367	1.4
Indiana	40,957	822	2.0
Kansas	15,818	334	2.1 <sup>2</sup>
Kentucky	21,553	269	1.2
Louisiana	44	0	0.0
Maryland	17,866	3,935	22.0
Massachusetts	52	3	5.8
Michigan	3,613	85	2.4
Missouri	100,802	1,609	1.6
New York	20,941	278	1.3
Ohio	79,348	1,731	2.2
Oklahoma	2,489	20	0.8
Pennsylvania	8,027	1,174	14.6
Tennessee	9,255	120	1.3
Texas	5,394	58	1.1
Virginia	1,489	28	1.9
Wisconsin	818	16	2.0
Total	339,953	10,850	3.2

<sup>1</sup> Data compiled from grain inspection records of the Grain Division, Bureau of Agricultural Economics.

<sup>2</sup> If 107 cars received at Salina, Kans., in 1935 and 1936 of which 63 were smutty are omitted, this figure becomes 1.7%. Shipments from the Pacific Northwest to Salina were heavy in those years.

Fifteen years ago the problem of breeding smut-resistant wheats seemed comparatively simple. At that time only a single race of stinking smut was known and it was thought that the varieties of wheat that were resistant to it would be safe from infection. Later studies have shown that there are numerous races or varieties of *Tilletia levis* and *T. tritici*, the fungi that cause bunt. The reactions of the varieties that differentiate 19 races are shown in Table X. From these results it may be noted that there are several pairs of varieties that together contain factors for resistance to all known races and it should be possible, theoretically, to cross these varieties and select hybrid lines resistant to all known races. This has already been accomplished. A selection (C.I. 10068-1) from a Hussar x Hohenheimer cross has been inoculated with all the known races and many collections from fields and has never had more than a trace of smut. Selections from a cross between Oro and a Turkey-Florence hybrid,



TABLE X

RELATIVE SUSCEPTIBILITY OF 10 DIFFERENTIAL HOSTS TO 11 PHYSIOLOGIC RACES OF *Tilletia tritici* AND 8 PHYSIOLOGIC RACES OF *T. levis*<sup>1</sup>

(R = 0%-10%; I = 11%-40%; S = 41%-100%)

Physiologic race No.	Hybrid 128 (C.I. 4512)	Ridit (C.I. 6703)	Oro (C.I. 8220)	Hohenheimer (C.I. 11458)	Hussar (C.I. 4843)	Albit (C.I. 8275)	Ulka (C.I. 11478)	Marquis (C.I. 3641)	Canus (C.I. 11637)	Minidum (C.I. 5296)
<i>Tilletia tritici</i>										
T-1	S	R	R	R	R	R	S	<b>R</b>	R	<b>R</b>
T-2	S	R	R	R	R	R	S	<b>R</b>	R	<b>S</b>
T-3	S	<b>R</b>	R	R	R	R	S	S	S	I
T-4	S	R	R	R	R	I	S	S	<b>R</b>	I
T-5	S	R	R	R	R	I	S	S	S	I
T-6	S	R	R	R	<b>R</b>	S	S	S	R	I
T-7	S	R	R	R	I	S	S	S	I	I
T-8	S	R	R	R	S	S	S	S	S	I
T-9	S	R	R	I	R	<b>R</b>	S	I	R	I
T-10 <sup>2</sup>	S	R	R	S	R	R	R	I	R	R
T-11	S	S	R	R	R	R	I	S	S	I
<i>Tilletia levis</i>										
L-1	S	R	R	R	R	R	S	I	R	I
L-2	S	R	R	R	R	R	S	S	R	I
L-3	S	R	R	R	R	R	S	S	S	I
L-4	S	R	R	R	R	S	S	I	<b>R</b>	I
L-5	S	R	R	R	<b>R</b>	S	S	S	S	I
L-6	S	R	R	R	I	S	S	S	S	I
L-7	S	R	R	R	S	S	S	I	S	I
L-8	S	R	S	R	R	R	S	S	S	I

<sup>1</sup> H. A. Rodenhiser and C. S. Holton, Physiologic races of *Tilletia tritici* and *T. levis*, J. Agr. Research 55: 485-496, Oct. 1, 1937.<sup>2</sup> Reactions that differentiate physiologic races are indicated by bold-face type.<sup>3</sup> The reaction of spring wheat differential hosts to this race was obtained in 1936 only. The results are therefore not strictly comparable with those recorded for the other races. The results are included, however, to indicate particularly the resistance of Ulka to race T-10.

which has the same reaction to races as Ridit, have also been highly resistant in all tests thus far made. Inoculum of at least three races, identified after the data in Table X were published, has been included in these tests. Resistance to the 22 known races appears to have been combined in these hybrids. They were developed in the western states and are not adapted for commercial production in the soft red winter region but it should be possible to transfer this resistance to adapted commercial types.

### Other Diseases

There are several other diseases that cause appreciable losses only in restricted areas and that should be given consideration in the improvement program for those areas. For example, the mosaic

disease causes heavy losses in some fields in southern Indiana and Illinois. A number of varieties of soft red winter wheat are resistant to this disease, while others are very susceptible. Leaf spot (*Septoria tritici*) and glume blotch (*S. nodorum*) cause heavy losses in some years, especially in the Atlantic Coastal Plains area. Scab has caused heavy losses in some years in the Corn Belt.

### Objective of the Breeding Program

The most common evaluation of wheat varieties in the past has been on the basis of yield. There usually are present one or more deleterious factors, such as winterkilling, lack of moisture, the Hessian fly, or one or more diseases such as leaf rust, stem rust, bunt, and loose smut, which reduce the yield. The objective of the wheat-improvement program is to lessen the fluctuation in production from year to year by reducing the toll taken by these crop hazards with respect both to yield and quality. This objective will most readily be attained by breeding into the widely adapted commercial varieties such characteristics as resistance to winterkilling, Hessian fly, lodging, and the various diseases.

## THE RELATION BETWEEN PROTEIN CONTENT AND STRENGTH OF GLUTEN-ENRICHED FLOURS<sup>1</sup>

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(Read at the Annual Meeting, May 1938)

A review of the literature dealing with the relation between protein content and loaf volume of flours produced from the same class of wheat reveals that many of the investigators have found a non-linear relationship. In the earlier studies, such as those of Bailey (1913), Stockham (1920), Shollenberger (1923), Bailey and Sherwood (1926), the increase in loaf volume per unit increase in protein content diminished until a protein level of from 15% to 16% was reached; in some cases further increases were accompanied by loaf-volume decreases. In the light of the more recent studies of Larmour (1931), Harris (1932) and Geddes and Larmour (1933), demonstrating the greater bromate response of the higher-protein flours, it appears probable that the true relative strengths of such flours, as reflected by loaf volume, were not revealed by the earlier baking methods; such a situation would naturally result in a non-linear regression. In some instances variations in

<sup>1</sup> Published as paper No. 148 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.

the "protein character" of the flours may also have been a factor, especially where flours of varying grade were involved in the comparisons.

The procedure reported by Aitken and Geddes (1937) for preparing dried gluten appeared to provide a means of securing a series of flours of any desired level in protein content without introducing differences in protein character, and the study reported here was undertaken to determine the relation between loaf volume and protein content over a wider range in the latter variable than has been possible heretofore.

### Experimental

Since any alteration in the strength-imparting properties of gluten as a result of its preparation would have an important bearing on the loaf volumes obtained with a series of flours containing increments of dried gluten, a preliminary study was undertaken to determine whether such protein-enriched flours gave baking results similar to normal flours of equivalent protein content. For this purpose, three high-grade Canadian hard red spring wheats were composited from envelope samples to yield long patent flours of approximately 12, 14, and 16% protein content; the individual samples in the blends were confined to the first three grades which comprise sound wheat of the Marquis type and were considered to be uniform in protein "quality."

Portions of each flour were employed to prepare powdered dried gluten according to the method described by Aitken and Geddes (1937) with the exception that the Dill and Alsberg (1924) solution was used for washing; the yield of dried gluten ranged between 17 and 22%, depending upon the protein content of the flours. Using the original flours and the dried gluten prepared therefrom, the series of protein-enriched flours listed in Table I was prepared and baked in random order, in comparison with the original flours, by the malt-phosphate-bromate (0.001%  $\text{KBrO}_3$ ) formula as outlined by Aitken and Geddes (1934) according to the A. A. C. C. procedure.

The data recorded in Table I show that the absorptions and loaf volumes of the gluten-enriched flours correspond very closely with those of the unenriched original flours of equivalent protein content, and indicate that the process employed for obtaining gluten in a dried fine granular state does not alter its strength-imparting characteristics to any apparent extent. The doughs from the gluten-treated flours were indistinguishable from the original flours of the same protein content as regards spring and general handling quality; also the external and internal characteristics of the loaves were closely similar.

In view of these results, dried gluten was prepared from a low-protein, experimentally milled hard red spring wheat flour; this was

TABLE I  
PROTEIN AND LOAF VOLUME DATA

Reference No.	Flour	Protein <sup>1</sup>	Absorption <sup>2</sup>	Loaf volume	Loaf volume per unit protein
A	Original, low protein	%	%	c.c.	c.c.
B	Original, medium protein	11.9	54.9	655	55
AA <sub>2</sub>	A + "A" dried gluten	13.8	57.0	728	53
AB <sub>2</sub>	A + "B" dried gluten	13.6	—	716	53
AC <sub>2</sub>	A + "C" dried gluten	13.9	—	725	52
		13.5	—	716	53
C	Original, high protein	15.7	59.0	774	49
AA <sub>3</sub>	A + "A" dried gluten	15.5	58.9	795	51
AB <sub>3</sub>	A + "B" dried gluten	15.5	—	786	51
AC <sub>3</sub>	A + "C" dried gluten	15.6	59.4	804	52
BA <sub>3</sub>	B + "A" dried gluten	15.7	59.2	781	50
BB <sub>3</sub>	B + "B" dried gluten	15.7	59.4	816	52
BC <sub>3</sub>	B + "C" dried gluten	15.8	59.6	816	52

<sup>1</sup> N  $\times$  5.7 on a 13.5% moisture basis. Protein content determined by a modification of the Kjeldahl-Gunning procedure.

<sup>2</sup> Determined by the Brabender farinograph (small mixer) at a dough consistency of 600 units. Results expressed on a 13.5% moisture basis.

added to the original flour in increasing proportions to provide a series of seven samples ranging in protein content from 10.5 to 22.7% in approximately 2% increments. The results of miscellaneous determinations on these flours are recorded in Table II, and it is of interest to note the increase in water-absorption and dough-development time

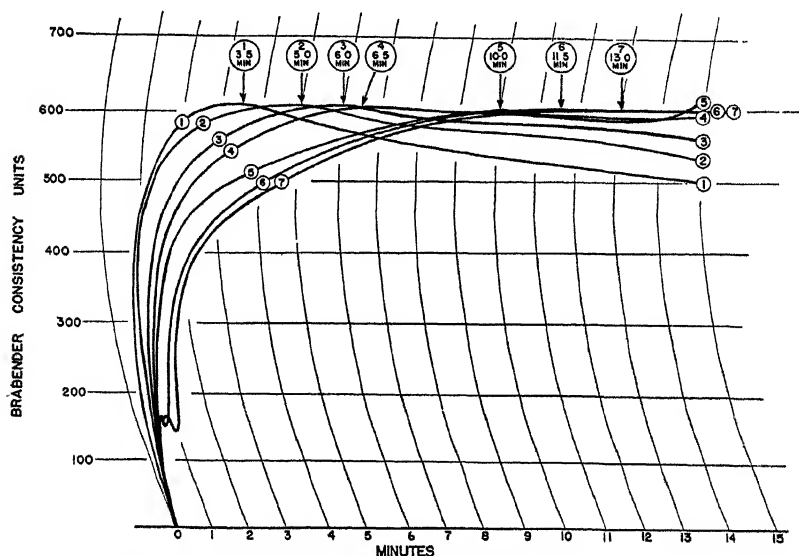


Fig. 1. Farinogram median lines illustrating effect of increasing protein content on type of curve.

with each increment of increase in protein content as revealed by the farinograph data. The changes in curve type are illustrated in Figure 1, in which lines drawn through the middle of the individual farinogram bands are reproduced; curve No. 1 resembles that of a medium-strength flour, while curves 2, 3, and 4 resemble those of strong Canadian Northern wheats; curves 5 and 6 have only rarely been encountered in this laboratory on samples of very high protein content, while No. 7 has never been obtained on a natural flour. These progressive changes in curve characteristics from that of a medium to that of a very strong flour indicate that protein content is an important factor in determining the type of farinogram.

TABLE II  
MISCELLANEOUS CHEMICAL AND BRABENDER FARINOGRAPH DATA

Chemical data (13.5% moisture basis)						Brabender farinograph data	
Flour No.	Protein content (N $\times$ 5.7)	Ash content	Diastatic activity (maltose per 10 g. flour)	Gassing power (gas from 25 g. flour after 6 hours)	Lipides	Absorption 13.5% M.B. (600 units)	Dough development time
	%	%	mg.	c.c.	%	%	min.
1	10.5	0.53	273	—	1.96	59.7	3.5
2	13.0	0.54	—	558	—	62.3	5.0
3	14.9	0.55	256	—	—	62.7	6.0
4	16.8	0.55	—	520	—	65.1	6.5
5	19.0	0.56	247	—	3.34	66.9	10.0
6	20.7	0.57	—	483	—	68.5	11.5
7	22.7	0.59	234	—	—	70.7	13.0
Dried gluten	64.8	0.75	124	—	9.98	—	—

Ash content determined by the Official A.O.A.C. procedure. Diastatic activity determined by the method outlined by Blish and Sandstedt (Cereal Chem. 10: 189-202, 1933). Gassing power determined as outlined by Bailey and Johnson (Cereal Chem. 1: 293-304, 1924). Lipide content determined as outlined in Cereal Laboratory Methods, 1935, p. 89.

For the purpose of determining comparative baking strength, the flours were baked according to the A. A. C. C. procedure at three levels of diastatic activity and four of potassium bromate, 0.1%  $\text{NH}_4\text{H}_2\text{PO}_4$  being added in all cases; in view of the large amount of material which would be required for replicate tests, single loaves were baked by each formula. The estimation of experimental error was made possible by designing an experiment in which protein level was confounded with days; each flour was baked by all twelve formulas on successive days, the baking formulas being randomized.

In addition to determining loaf volume, the loaves were scored for external and internal characteristics. The loaves exhibited a trend from "green" or underfermented to "old" or overfermented character-

istics with increasing increments of bromate which was particularly noticeable in the instance of the low-protein flours.

The loaf-volume results are recorded in Table III and a var analysis of these data is given in Table IV. Highly significant differences exist between the volumes for different protein levels and bromate treatments but the differences due to malt additions are not significant indicating that the untreated flour was sufficiently high in diastase activity (273 units) to eliminate gas production as a factor affecting loaf volume. The highly significant interaction of protein content and bromate level is a reflection of the increased tolerance to bromate with increasing protein content.

TABLE III  
LOAF-VOLUME DATA

Flour protein	No malt				0.5% malt			
	No KBrO <sub>3</sub> (B <sub>0</sub> M <sub>0</sub> )	1 mg. KBrO <sub>3</sub> (B <sub>1</sub> M <sub>0</sub> )	2 mg. KBrO <sub>3</sub> (B <sub>2</sub> M <sub>0</sub> )	3 mg. KBrO <sub>3</sub> (B <sub>3</sub> M <sub>0</sub> )	No KBrO <sub>3</sub> (B <sub>0</sub> M <sub>1</sub> )	1 mg. KBrO <sub>3</sub> (B <sub>1</sub> M <sub>1</sub> )	2 mg. KBrO <sub>3</sub> (B <sub>2</sub> M <sub>1</sub> )	3 mg. KBrO <sub>3</sub> (B <sub>3</sub> M <sub>1</sub> )
%	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
10.5	711	655	552	458	720	716	533	464
13.0	697	706	665	495	711	767	650	517
14.9	762	823	702	543	772	879	669	555
16.8	776	926	781	623	781	940	809	618
19.0	898	1058	922	781	907	1044	879	773
20.7	922	1120	1110	874	988	1166	1039	882
22.7	1091	1269	1297	935	1100	1246	1241	935

Flour protein	1.0% malt				Mean, all malt levels			
	No KBrO <sub>3</sub> (B <sub>0</sub> M <sub>2</sub> )	1 mg. KBrO <sub>3</sub> (B <sub>1</sub> M <sub>2</sub> )	2 mg. KBrO <sub>3</sub> (B <sub>2</sub> M <sub>2</sub> )	3 mg. KBrO <sub>3</sub> (B <sub>3</sub> M <sub>2</sub> )	No KBrO <sub>3</sub>	1 mg. KBrO <sub>3</sub>	2 mg. KBrO <sub>3</sub>	3 mg. KBrO <sub>3</sub>
%	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
10.5	725	744	557	500	719	715	547	469
13.0	730	748	664	524	713	740	660	517
14.9	804	856	702	557	779	853	691	555
16.8	823	959	800	637	793	942	797	618
19.0	949	1072	898	804	918	1058	900	773
20.7	967	1138	1067	903	959	1141	1072	882
22.7	1072	1260	1119	964	1088	1258	1219	935

The regression coefficients for loaf volume on protein are recorded for each formula in Table V, together with those for the means of all malt treatments combined for each level of bromate; as a measure of the significance of the differences in the regression coefficients, the

TABLE IV  
ANALYSIS OF VARIANCE FOR LOAF-VOLUME DATA

ariance due to:	D.F.	Variance	F	5% point
een bromate levels	3	277164.77	126.9	4.76
een malt levels	2	1590.08	—	—
action:				
Malts × bromates	6	2184.75	4.15	2.36
(error for above)				
een protein levels	6	439432.08	60.68	2.66
ractions:				
Protein × malts	12	984.30	1.87	2.03
Protein × bromates	18	7241.75	13.77	1.93
(error for protein)				
Protein × malts × bromates	36	525.92	—	—
(error for interactions)				
Total	83	—	—	—

ults of covariance analyses are also recorded. Significant differences exist between the regression coefficients for the various bromate levels, those for the 0.001% and 0.002% being the highest; an analysis of variance showed that these two regressions are not significantly different. The regressions for the combined volumes of all malt levels at each bromate treatment are shown graphically in Figure 2. While

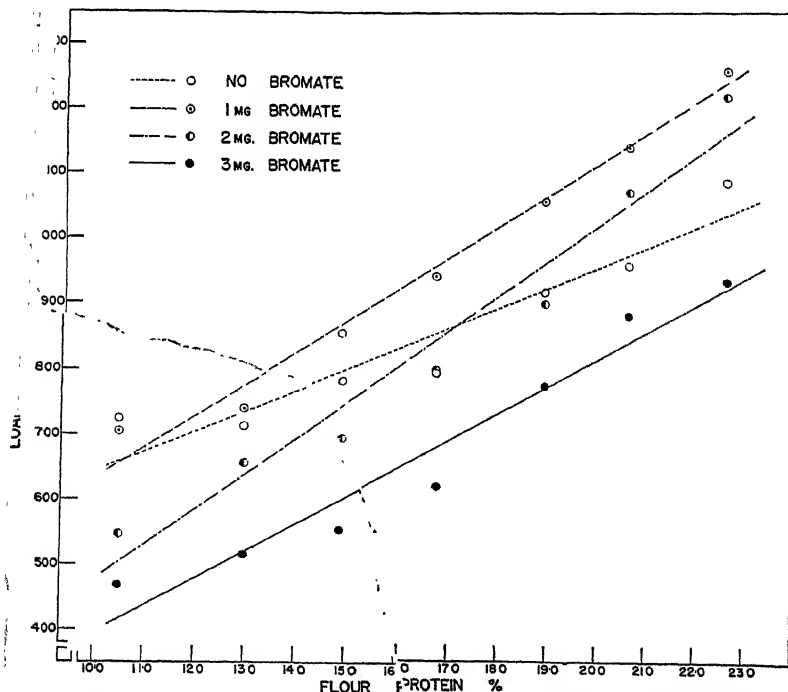


Fig. 2. Regression of loaf volume on flour protein as influenced by potassium bromate content.

there are insufficient data to permit an accurate test of linearity, obvious from the graph that the basic and 0.001% bromate volumes bear a linear relation to protein content but the differentiation between the flours is decidedly better for the latter procedure.

TABLE V  
REGRESSION COEFFICIENTS AND COVARIANCE ANALYSES FOR LOAF VOLUME C  
PROTEIN

Individual formulas			All malt levels for different bromate treatments	
Flour treatment		Regression coefficient	Bromate treatment	Regression coefficient
Bromate	Malt			
mg.	%	$b_{yz}$	mg.	$b_{yz}$
0	0.0 (B <sub>0</sub> M <sub>0</sub> )	30.48	B <sub>0</sub>	30.73
1	0.0 (B <sub>1</sub> M <sub>0</sub> )	51.60	B <sub>1</sub>	47.32
2	0.0 (B <sub>2</sub> M <sub>0</sub> )	59.25	B <sub>2</sub>	54.03
3	0.0 (B <sub>3</sub> M <sub>0</sub> )	43.03	B <sub>3</sub>	41.62
0	0.5 (B <sub>0</sub> M <sub>1</sub> )	32.27		
1	0.5 (B <sub>1</sub> M <sub>1</sub> )	45.29		
2	0.5 (B <sub>2</sub> M <sub>1</sub> )	55.14		
3	0.5 (B <sub>3</sub> M <sub>1</sub> )	39.40		
0	1.0 (B <sub>0</sub> M <sub>2</sub> )	29.44		
1	1.0 (B <sub>1</sub> M <sub>2</sub> )	45.07		
2	1.0 (B <sub>2</sub> M <sub>2</sub> )	47.72		
3	1.0 (B <sub>3</sub> M <sub>2</sub> )	42.44		

## COVARIANCE ANALYSES

Variance due to:	Individual formulas				All malt levels			
	D.F.	Variance	F	5% pt.	D.F.	Variance	F	5% pt.
Total	71	—	—	—	71	—	—	—
Within regressions	60	2,311.52	—	—	68	2,457.09	—	—
Between regressions	11	10,071.58	4.36	1.95	3	32,888.30	13.38	2.74

The results of these experiments show that in testing a series of flours of similar protein character where gas production is not a limiting factor, the 0.001% bromate formula in particular yields volumes which are essential as a measure of the protein content. Also, since potassium bromate influences the colloidal characteristics of the dough undergoing fermentation and different bromate levels give different volumes, it follows that when protein content and gas production are held constant, variations in loaf volume are a function of differences in colloidal characteristics.



### Summary

Dried powdered gluten may be prepared which has similar strength-starting properties to those of the original flour protein.

Seven flours ranging in protein content from 10.5% to 22.7%, obtained by enriching the lowest-protein flour with dried gluten prepared therefrom, were submitted to Brabender farinogram tests and also baked by twelve formulas comprising three levels of diastatic activity and four of potassium bromate.

The farinograms showed an increase in water-absorption and dough-development time and a decrease in "weakening area" with increasing protein content, the curves indicating transformation from a medium strong to exceedingly strong flours.

With increasing increments of bromate the loaves exhibited a trend from under- to over-fermented characteristics.

Significant differences existed in the regression coefficients for loaf volume on protein for the various bromate levels, those for the 0.001% and 0.002% being the highest. Loaf volumes by the basic and 0.001%  $\text{KBrO}_3$  formulas bore a linear relation to protein content over the entire range but the latter gave greater differentiation.

With flours of similar protein character where gas production is not limiting factor, loaf volume is essentially a measure of protein content, and *vice versa* when a baking formula containing sufficient potassium bromate (0.001 to 0.002%) to yield approximately optimum volumes is employed.

### Acknowledgments

The authors are indebted to C. H. Goulden for advice in designing the experiment, and to Nancy Milton for her work in connection with the statistical analyses.

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## EXPERIMENTS ON THE SEPARATION OF SELENIUM FROM ITS COMBINATION WITH PROTEINS IN GRAIN

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In the course of investigations by Smith, Westfall, and Stohlman (1938) on the fate of selenium in the animal organism, it was noted that under suitable conditions considerable amounts of selenium could be split off from protein by bromine in hydrobromic acid at room temperature without any grossly apparent dissolution of the protein. The results of earlier studies by Franke and Painter (1936), Painter and Franke (1936), Horn, Nelson, and Jones (1936), and Jones, Horn, and Gersdorff (1937), seemed to indicate that naturally occurring selenium in grain is an integral part of the protein and that it could not be removed by any procedure short of its complete disintegration by acid hydrolysis. It seemed desirable therefore to investigate the matter further in some detail.

The present experiments were carried out for the most part upon selenium-bearing wheat protein prepared from seleniferous wheat by a method similar to that described by Franke and Moxon (1934).<sup>2</sup> This material contained 50 ppm. selenium and was made from wheat carrying 10 ppm. selenium. Some of the experiments were also performed upon similarly prepared protein derived from oat flour, upon gluten made in this laboratory by leaching out part of the starch from selenium-bearing wheat flour, and also upon selenium-bearing whole-wheat and whole-oat flour. The technique for selenium removal consisted of bringing a weighed amount of the material in contact with the "extraction mixture" by frequent stirring, and at the end of the observation period the

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<sup>2</sup> We are indebted to A. L. Moxon of the South Dakota State College for a generous supply of this material.

selenium was determined in an aliquot of the filtered or centrifuged solution. The selenium was estimated nephelometrically as previously described (Smith, Westfall, and Stohlman, 1938; Smith, Franke, and Westfall, 1936).

### Results

The data presented in Tables I to VII show the results obtained. Table I shows that 24 hours' contact of selenium-bearing protein with the oxidizing agents, bromine and hydrogen peroxide, effects a separation of the selenium. The separation is quantitative if the conditions are adequate. For the effective separation of selenium with bromine, hydrobromic acid appears to be essential though the latter alone is wholly ineffective. The presence of sufficient trichloroacetic acid to prevent solution of appreciable amounts of protein does not affect the extent of selenium separation. With an extraction mixture of 1% bromine and 10% hydrobromic acid, the minimum volume in relation to the protein to effect complete separation of selenium in 24 hours at room temperature is shown in Table II, which indicates that upwards of 20 c.c. are required per gram of material.

TABLE I

THE SEPARATION OF SELENIUM FROM WHEAT PROTEIN BY OXIDIZING AGENTS  
(Two g. protein, 100 c.c. aqueous extraction mixture, 24 hours' contact at room temperature)

Trichloroacetic acid	Hydrobromic acid	Oxidizing agent	Selenium removed <sup>1</sup>
%	%		%
16	10	1% bromine	100
0	10	1% bromine	91
0	0	1% bromine	44
16	0	1% bromine	32
0	10	None	Trace
0	0	None	Trace
16	0	5% H <sub>2</sub> O <sub>2</sub>	32
16	0	15% H <sub>2</sub> O <sub>2</sub>	103

<sup>1</sup> In this and subsequent tables percent removed is based on total selenium found by complete oxidation by the open beaker wet ashing method (Smith, Westfall, and Stohlman, 1938; Smith, Franke, and Westfall, 1936).

TABLE II

EFFECT OF VOLUME OF EXTRACTION MIXTURE ON THE YIELD OF SELENIUM IN ITS SEPARATION FROM WHEAT PROTEIN

(One % bromine in 10% hydrobromic acid, 24 hours' contact at room temperature)

Volume	Selenium removed
c.c.	%
50	101
20	75
10	54
5	3

In Table III the minimum concentration of bromine to effect complete separation of selenium is shown, indicating that a concentration of at least 0.5% is needed. In like manner the minimum concentration of hydrobromic acid appears to be 10%, as shown in Table IV. That time and temperature of the reaction are also factors in the splitting

TABLE III

EFFECT OF CONCENTRATION OF BROMINE ON THE SEPARATION OF SELENIUM FROM WHEAT PROTEIN

(Fifty volumes of extraction mixture consisting of 16% trichloroacetic acid, 10% hydrobromic acid, and bromine as indicated, 24 hours' contact at room temperature)

Concentration of bromine	Selenium removed
%	%
0.125	20
0.25	60
0.50	96
1.00	100

TABLE IV

ACCELERATING EFFECT OF HYDROBROMIC ACID ON THE RATE OF SEPARATION OF SELENIUM FROM WHEAT PROTEIN BY BROMINE

(Fifty volumes of extraction mixture consisting of 1% bromine in hydrobromic acid as indicated, 24 hours' contact at room temperature)

Concentration of hydrobromic acid	Selenium removed
%	%
0.0	44
2.5	62
5.0	76
10.0	98

TABLE V

EFFECT OF TIME AND TEMPERATURE ON THE RATE OF SEPARATION OF SELENIUM FROM WHEAT PROTEIN BY BROMINE IN HYDROBROMIC ACID

(Fifty volumes of extraction mixture consisting of 1% bromine and 10% hydrobromic acid)

Time	Temperature, Centigrade	Selenium removed
Hrs.		%
1½	25	22
3	25	44
6	25	62
24	20-25	100
6	0	5
3	40	80
6	40	87

TABLE VI

EFFECT OF CONCENTRATION OF  $\text{H}_2\text{O}_2$  ON THE RATE OF SEPARATION OF SELENIUM FROM WHEAT PROTEIN

(Fifty volumes of extraction mixture, 24 hours' contact at room temperature)

Hydrogen peroxide	Trichloroacetic acid	Selenium removed
%	%	%
6	16	32
6	0	31
12	0	40
12	0	46
18	16	100
18	0	92

TABLE VII

SEPARATION OF SELENIUM FROM ITS COMBINATION IN GRAIN AND IN GRAIN PROTEIN

(One % bromine in 10% hydrobromic acid, 24 hours' contact at room temperature, 50 c.c. per g. material)

No.	Sample	Selenium content	Selenium removed
		Mg. %	%
1	Oats	0.7	65
2	Oats	0.9	80
3	Oats	1.4	36
4	Oats	1.0	25
5	Wheat	1.0	36
6	Wheat	1.1	60
7	Wheat	1.9	70
8	Oat protein	1.2	100
9	Wheat gluten	3.8	95
10	Wheat protein, lot 1	5.0	95
11	Wheat protein, lot 2	5.1	106
12	Wheat protein, lot 3	5.0	93
13	Wheat protein, lot 4	4.7	100

off of selenium from seleniferous protein is clearly shown in Table V.

In Table VI the concentration of hydrogen peroxide most effective in removing selenium is shown, and this appears to be 18% if used in the proportion of 50 c.c. per gram of material.

In order to ascertain the applicability of the above procedures to the selenium in whole grain, several samples of ground selenium-bearing oats and wheat were treated with bromine in hydrobromic acid or with hydrogen peroxide under the optimum conditions and the percentage of selenium so removed determined. The results as shown in Table VII indicate that selenium can be separated from whole grain as well as from grain protein, though removal in the former case is often incon-

plete.<sup>3</sup> Samples 3, 4, 5, and 6, which gave low values, were subsequently treated in the same manner with the bromine increased to a concentration of 3% with better results, the percentages of selenium obtained having been increased to 62, 88, 50, and 81 respectively. In another experiment samples 4 and 5 were treated with 18% hydrogen peroxide, and the percentages of selenium recovered were 100 and 90, respectively. It seems probable that some of the low recoveries of selenium from whole grain when treated with bromine in hydrobromic acid may be due in part to interference of starch with the recovery of the volatile bromide of selenium in the process of distillation.

### Discussion

It is not implied that these results are suggested as a means of detoxifying selenium-bearing grain. Indeed wheat gluten treated with bromine as described here becomes highly toxic and apparently out of all proportion to the bromine it retains. Wheat gluten treated with hydrogen peroxide in a manner similar to that used for the removal of selenium is apparently devoid of toxicity as judged by feeding experiments in rats, but it is probable that such treatment may affect its nutritional value. We report these experiments merely to show that selenium can be split off from grain and grain protein under suitable conditions without disintegrating the protein.

It is reasonable to inquire what light, if any, the present experiments throw on the chemical nature of the selenium in grain protein. Blumenthal and Clarke (1935) have succeeded in splitting off a fraction of the protein sulfur as inorganic sulfate with bromine, which they consider as neither cystine nor methionine; and Smith and Harris (1936) showed that cystine sulfur in sheep's wool is attacked even by 3% hydrogen peroxide. In our experiments at least a portion of the selenium separated from protein with bromine in hydrobromic acid can be precipitated as elementary selenium by reduction with sulfur dioxide and hydroxylamine hydrochloride.

Similarly the selenium split off with hydrogen peroxide can also be precipitated in its elementary form by reduction after a preliminary brief treatment with bromine in hydrobromic acid. The selenium thus appears to behave like the inorganic selenite or selenate. This, however, does not exclude the possibility of its being an organic compound of selenium, either as it naturally occurs in protein or as some derivative, for we have observed that the very labile selenium of the organic compound diseleno diacetic acid<sup>4</sup> can also be removed quantitatively by

<sup>3</sup> Curl and Osborn (J. Assoc. Official Agr. Chem. 21: 228-235, 1938) have reported the extraction of 81% of the selenium from a mixed sample of sunflower seed, wheat, barley, and oats by refluxing 1½ hours with 15% bromine in 48% hydrobromic acid. Their mixed sample contained 16.5 mg. percent selenium.

<sup>4</sup> Diseleno diacetic acid,  $\text{HOOC}\cdot\text{CH}_2\cdot\text{Se}\cdot\text{Se}\cdot\text{CH}_2\cdot\text{COOH}$ , was kindly supplied by H. P. Ward of the Catholic University of America.

reduction with sulfur dioxide and hydroxylamine hydrochloride after a brief preliminary treatment with bromine in hydrobromic acid. Taking everything into consideration it appears likely that the major portion of the selenium split off from the protein by the two procedures described is probably inorganic and gives no clue to the chemical nature of its precursor in the protein other than that it is moderately labile.

A series of analyses was made to determine the nitrogen and sulfur content of the selenium-containing extracts obtained by deselenizing two samples of seleniferous wheat protein.<sup>5</sup> For comparison analyses were also made for the nitrogen and sulfur content of similar extracts obtained from a sample of non-seleniferous wheat gluten. The results, which are summarized in Table VIII, show that all the extracts con-

TABLE VIII  
NITROGEN AND SULFUR CONTENT OF PROTEIN EXTRACTS OBTAINED  
IN THE PROCESS OF DESELENIZATION

Description of preparation	N <sup>1</sup>	S <sup>1</sup>	Percent of total	
			N	S
	%	%	%	%
1. <i>Extracts of selenium-bearing wheat gluten containing 4.26% N and 0.24% S</i>				
Trichloroacetic acid extract	0.35	0.03	8.2	12.5
Trichloroacetic-bromine-hydrobromic acid extract	0.91	0.09	21.4	37.0
Trichloroacetic acid-hydrogen peroxide extract	1.12	0.16	26.3	67.0
2. <i>Extracts of selenium-bearing wheat protein containing 13.70% N and 0.93% S</i>				
Trichloroacetic acid extract	1.12	0.06	8.2	6.5
Trichloroacetic-bromine-hydrobromic acid extract	1.97	0.23	14.4	24.7
Trichloroacetic acid—hydrogen peroxide extract	3.12	0.24	22.8	25.9
Bromine-hydrobromic acid extract	2.08	0.30	15.2	32.0
Hydrobromic acid extract	3.46	0.11	25.0	11.8
3. <i>Extracts of non-seleniferous wheat gluten containing 14.40% N and 0.74% S</i>				
Trichloroacetic acid extract	1.05	0.04	7.1	5.4
Trichloroacetic-bromine-hydrobromic acid extract	2.27	0.14	15.8	19.0
Trichloroacetic acid-hydrogen peroxide extract	3.24	0.17	23.2	23.0

<sup>1</sup> Nitrogen by the Kjeldahl method; sulfur in extracts by the Benedict-Denis, in proteins by the Parr bomb method.

tained some nitrogen and sulfur. Since the selenium-free trichloroacetic acid extracts contained as much as 8% of the total nitrogen and 12% of the total sulfur, not more than about 6% of the total nitrogen nor more than 18% of the total sulfur could be intimately associated with the selenium of the protein. Even this becomes doubtful in view of the fact that a hydrobromic acid extract of a seleniferous protein con-

<sup>5</sup> For the nitrogen determinations thanks are due to E. Elvove and C. G. Remsburg of this Institute. At E. Elvove's suggestion the excess bromine was reduced with zinc dust prior to digestion since bromine may cause loss of nitrogen.

taining not more than a trace of its selenium contained actually more nitrogen than the corresponding bromine-hydrobromic acid extract which had removed all the protein selenium. However this increase probably represents simply some acid-soluble fraction, since some protein is precipitated from the hydrobromic acid extract on subsequent addition of bromine. Since a portion of the nitrogen and sulfur is removed by both oxidizing agents and especially since the sulfur in these is higher than in the hydrobromic acid extract it would seem not improbable that the breaking of some sulfur linkages may accompany the selenium extractions. Nevertheless extracts of non-seleniferous wheat gluten contained approximately as much of its total nitrogen and sulfur as did the extracts of the selenium-bearing proteins.

### Summary

The selenium which naturally occurs in grain protein can be removed quantitatively under suitable conditions with (1) bromine in hydrobromic acid, or (2) hydrogen peroxide. While this entails no grossly apparent hydrolysis of the protein nor its disintegration, there is some possibility that it is accompanied by the removal of a certain fraction of nitrogen and sulfur.

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# EVALUATION OF YEAST ACTIVITY BY MEANS OF THE SANDSTEDT-BLISH PRESSURE METER<sup>1</sup>

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The testing of yeast used in commercial bread production for uniformity of strength, as well as the testing of various brands of yeast for comparative gas production activities, is one of the routine functions of the bakery service laboratory. While other qualities of yeast, such as proteolytic activity and rope spore count, are of importance in determining the commercial value of yeast, the rate of gas production and the total amount of gas produced over a period of time comparable to normal fermentation are of major importance and should be known independently of other characteristics.

The strength of yeast used in bread making is primarily dependent on the rate with which it produces carbon dioxide when an adequate supply of fermentable carbohydrate is available. Cook and Malloch (1930) outlined a method for determining the carbon dioxide produced from maltose by a definite quantity of yeast acting in a liquid medium. This required adjustment of pH to that of a normal fermenting dough and constant agitation of the fermenting medium by mechanical shaking. This method was, however, open to the objection cited by the authors that the relative efficiencies in dough of two strains of yeast are not necessarily the same as in liquid media.

The shortcomings of a method which does not test yeast in the medium in which it is used, are recognized by the yeast manufacturers themselves, who control the uniformity of their product by making a dough of flour, water, fermentable carbohydrate (sugar) and shortening of the consistency of normal bread dough and recording the time required for it to reach a definite height. Note that this is what is known as a straight dough. Other methods along the lines of testing yeast *in situ* depend on measuring the amount of gas evolved from a fermenting dough, including that required for raising the dough. Many such methods have been proposed in the literature. Bailey and Johnson (1924) measured the gas produced on fermentation by collecting the gas in an inverted burette. C. W. Brabender (1934) developed an apparatus known as the fermentograph which is so arranged that the amount of carbon dioxide given off by the fermenting dough displaces an equal volume of water, the weight of which is recorded automatically by a recording mechanism.

<sup>1</sup> Sub-committee report, 1937-38 Committee on Methods of Analysis.

Obviously this method of measuring the gas-producing activity of a dough can be utilized for determining the rates of activity of various types and brands of yeast. Near and Sullivan (1935) published their observations of the variability in gassing strength of several brands of yeast, as measured by this apparatus. It was their opinion that corresponding periods of rate of gas production could be correlated very well with the gas produced during the proofing period of the baking test.

The manometric method for determining gas-producing capacity of flour designed by Blish, Sandstedt, and Astleford (1932) and refined by Sandstedt and Blish (1934) is widely used for commercial control of "gassing" power of flour without, however, sufficient emphasis on the different activities of various types of yeast. The work we have done shows clearly that there is sufficient difference in activity between different types of yeasts to make it important to specify the source of the yeast used in any series of collaborative tests on gassing strength of flour.

A comparative study of the Bailey-Johnson, fermentograph, and Sandstedt-Blish manometric methods for determining gassing power of flour was made by Eva, Geddes, and Frisell (1937). They reached the conclusion that there was little to choose between the three methods from the standpoint of utility, and that the adoption of any particular method could be based on other considerations.

The general acceptance of the Sandstedt-Blish pressure meter as finally manufactured as a convenient apparatus for measuring gas-producing activity of flours led to an investigation of its utility for testing the uniformity and relative activity of yeast. This was one of the projects of the Methods Committee for 1937.

### Experimental Work

In the following experiments yeasts are referred to as A, B, C, etc. No comparison can be made between the strengths of the yeasts as given in the various tables, as the same letters do not always identify the same yeast.

The first tests were designed to determine if a normally diastated flour could be used without any source of additional fermentable carbohydrates. The method was the same as used in determining the gassing power of a flour. Ten g. of flour was mixed with 0.3 g. of yeast (3% based on flour) and 7 c.c. water and the dough allowed to ferment at 30° C. in a thermostatically controlled water bath. Readings were made of both the upper and lower levels of the mercury column and the difference recorded as millimeters of pressure. We found that the accuracy of this determination was increased by releasing the

TABLE I  
GAS EVOLVED FROM DIASTATED FLOUR

Time	Rate per hour		Total gas	
	Yeast A	Yeast B	Yeast A	Yeast B
<i>hrs.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	88.2	84.0	88.2	84.0
2	151.5	133.9	239.7	217.9
3	135.5	137.0	375.2	354.9
4	47.3	59.5	422.5	414.4
5	28.3	30.2	450.8	444.6
6	19.9	20.4	470.7	465.0

pressure at the end of each hour. The gas must be allowed to escape slowly so that the cooling effect due to adiabatic expansion does not affect the reading.

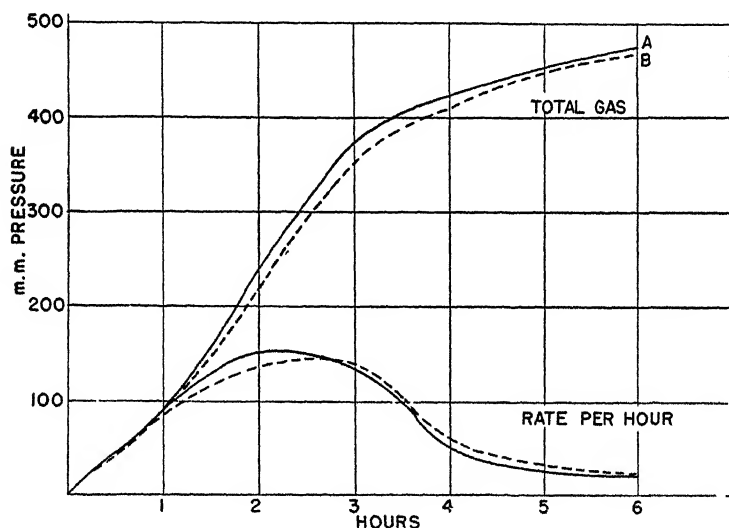


Fig. 1. Gas production with yeasts A and B and a diastated flour (no sugar).

As indicated by the results in Table I, and shown in Figure 1, while yeasts may vary in activity during the first hour or two of fermentation when there is an oversupply of carbohydrate, at later periods the rate of production of fermentable carbohydrates by diastatic activity is the limiting factor in gas production. Thus, a yeast which ferments maltose slowly may produce as much total gas or even more at the end of the fermentation period as the one more active the first few hours. This observation is of practical interest, as the conditions of the method approximate those existing in a bread sponge.

This illustrates the importance of specifying the kind of yeast used in the determination of gassing power. Thus there is considerable difference in the gas produced per hour by the two yeasts until the fifth hour. Two laboratories using the same flour would not agree on this figure unless the fifth or sixth hour were taken as the measure of diastatic activity.

In testing yeast for use in sponge fermentation it would seem advisable to test it without addition of sucrose. However, even with a flour of good diastatic capacity the activity of the yeast will be limited in the later stages to the maltose produced by diastatic action and the ability to ferment maltose, and misleading conclusions regarding the strength of the yeast may be reached if the results are not properly interpreted.

In the dough stage of the sponge process, excess sucrose is present and it is important therefore to know how the yeast will behave under such conditions. Also if yeast is to be used in straight doughs it would seem advisable to test it in the presence of sucrose. We have, therefore, experimented with adding sucrose in varying amounts, using the same method described above with the results shown in Table II and in Figure 2. The percentage of sugar was based on the flour.

TABLE II  
EFFECT OF ADDING SUCROSE TO DIASTATED FLOUR

Time	Sugar			
	0	2.5%	5.0%	7.5%
<i>hrs.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	85	114	109	102
2	137	127	138	139
3	140	113	117	127
4	55	98	97	114
5	34	81	86	101
6	25	60	85	96
Total	476	593	632	679

Table II shows that the production of gas from the dough fermenting in the pressure meter drops off rapidly after three hours when no sucrose is present. This is also shown in Table I and Figure 1. Therefore, if any information is desired regarding the activity of the yeast in the presence of excess carbohydrate after that time, fermenting material from an additional source must be added. After many experiments we selected 0.3 g. or 3%, based on the flour, of sugar, as giving us all the data normally required on the relative activity of yeast in the presence of sucrose. It is interesting to note that the

rate of gas production is faster during the third hour when no sugar is present than at any other time.

We also investigated the advisability of using maltose in place of sucrose, as this sugar is the one normally present in a fermenting

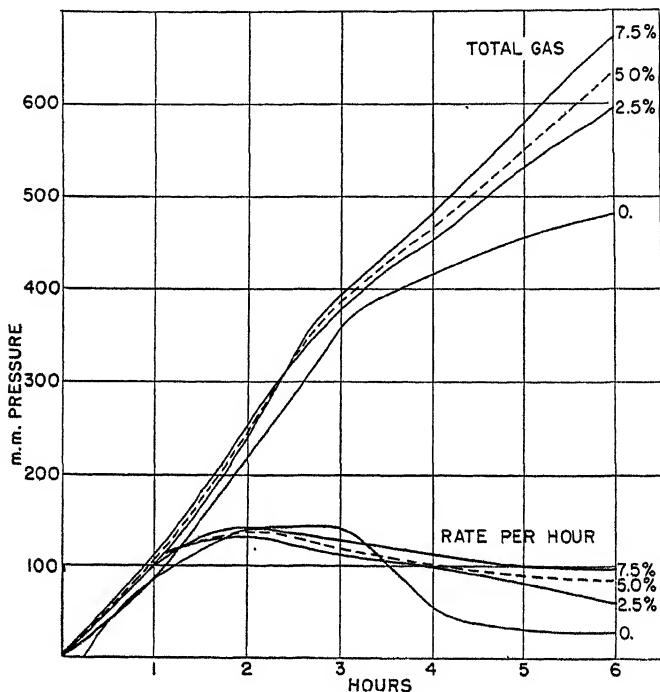


Fig. 2. Effect of added sucrose (10 g. of diastatic flour and 3% yeast).

sponge as a result of diastatic action of the flour or malt supplement added by the baker. We show in Table III a comparison of the two yeasts tested by the pressure-meter method in the absence of added sucrose, with 3% sucrose, and with 3% maltose.<sup>2</sup>

Yeast B was slower than A in producing gas the first two hours from both the no-sugar and the maltose dough. It produced less gas from maltose sugar at the end of 4 hours, which in our work covers the sponge fermentation time, but produced as much gas in 4 hours as A in the dough without added sugar. A review of the sucrose fermentation is interesting, because it indicates that during the first hour of fermentation the two yeasts are the same but that yeast B is slower the second hour. From baking tests we have made, the rate of gas

<sup>2</sup> Difco Standardized, Difco Laboratory, Detroit.

production from the sucrose dough appears to influence the pan proofing time of the sponge-dough process.

TABLE III  
COMPARISON OF YEASTS USING DIFFERENT SUGARS

Time	No sugar		0.3 g. sucrose		0.3 g. maltose	
	A	B	A	B	A	B
<i>hrs.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	94	81	118	118	88	80
2	136	125	129	113	126	114
3	120	124	129	121	144	135
4	47	69	96	91	131	125
5	24	31	85	85	83	95
6	23	24	63	71	50	70
Total	444	454	620	599	622	619
Total—4 hrs.	397	399	472	443	489	454

As the addition of maltose did not reveal any differences between the activities of yeasts in our tests other than shown by the no-sugar dough we continued our investigations with the no-sugar and sucrose doughs. We also studied the effect of absorption, varying it from 50% to 100% based on the flour, with results shown in Table IV.

TABLE IV  
EFFECT OF ABSORPTION ON GAS PRODUCTION—0.3 GRAM SUCROSE

Time	Absorption		
	50%	70%	100%
<i>hrs.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	88	90	85
2	129	140	148
3	108	131	143
4	35	41	45
5	33	32	35
6	14	17	20

The softer doughs generate more gas per hour than the stiffer ones but there appeared to be no physical advantage over the dough made with 7 c.c. or 70% water. This amount we found convenient. On the other hand the stiffer (50%) dough was harder to mix. We therefore continued with 7 c.c. of water and recommend it as a convenient amount to use. Further, this is the absorption suggested by Sandstedt and Blish for the determination of gassing power of flour and is the absorption normally encountered in sponge and dough fermentation.

The pressure-meter method can be used very satisfactorily for

determining the uniformity of any one type of yeast. It may be used with or without sugar for that purpose. Once the characteristics of any one type of yeast have been determined in terms of pressure-meter readings per hour, a standard can be set up which shipments can be expected to match with a reasonable degree of accuracy. We

TABLE V

PRESSURE-METER READINGS ON ONE BRAND OF YEAST IN PRESENCE OF SUCROSE

Weeks	Hours					
	1	2	3	4	5	6
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	121	128	97	93	92	73
2	110	133	116	89	89	63
3	117	132	115	96	89	74
4	120	114	116	97	85	70
5	102	133	117	99	80	65
6	121	142	108	100	80	68
7	115	131	111	101	81	70
8	117	130	121	96	78	64
9	120	132	114	103	79	63
10	118	132	112	94	80	72
Average	116	131	113	97	83	68

show in Table V the results obtained on one brand of yeast in the presence of sucrose over a period of ten weeks. The figures shown are the average of triplicate determinations.

TABLE VI

PRESSURE-METER READINGS—NO SUGAR

Weeks	Hours					
	1	2	3	4	5	6
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	75	123	137	61	34	20
2	78	126	132	60	33	19
3	87	143	135	57	28	20
4	86	146	139	59	28	19
5	83	135	139	63	27	19
6	89	131	134	57	30	23
7	90	131	137	60	31	23
Average	84	134	137	60	30	20

Table VI shows results of another yeast in absence of sucrose. This yeast was the same as yeast B in Table I. The data illustrate the uniformity with which yeast is delivered to the baker. They also show clearly that the diastatic activity of a flour is the limiting factor

duction when no added sugar is present, and verify the results of Sandstedt and Blüsch (1934) that yeast variability with type of yeast is insignificant in determining gassing power if it is fresh.

It was made to determine whether the method could be used to compare the various brands of yeast gave interesting results. Thus data for different yeasts in the presence of sugar are given in Table VII.

TABLE VII  
COMPARISON OF YEASTS—3 GRAMS SUGAR

Yeast	Hours						Total
	1	2	3	4	5	6	
	mm.	mm.	mm.	mm.	mm.	mm.	
A	121	149	113	99	88	73	643
B	110	140	106	91	75	67	589
C	84	131	101	93	88	85	579
D	102	125	87	79	74	64	531
E	118	135	128	116	95	62	662

These data present some interesting differences, clearly showing why it is necessary to run the test over at least 5 hours in order to get information on the activity of a yeast and its value from the standpoint of gassing power. Thus yeast A is very active throughout the entire period of the test. Yeast C shows a slow start but a well-sustained activity during the later period of the test. Yeast D starts out well but shows a very decided decrease in activity between the second and third hour. Thus if the test had been run only two hours or if the yeast had been tested in a medium consisting of low percentage of yeast and with salt present, different results would have been obtained.

TABLE VIII  
COMPARISON OF YEASTS IN GAS PRODUCTION PER HOUR

Time	Sugar			No sugar		
	A	B	C	A	B	C
hrs.	mm.	mm.	mm.	mm.	mm.	mm.
1	126	118	127	97	120	85
2	127	135	127	136	139	128
3	115	128	121	119	116	132
4	102	116	104	45	43	65
5	87	94	89	28	28	35
6	58	59	71	21	22	25
Total	613	650	639	446	468	470



In another series of tests several brands of yeast were run without sugar with the results shown in Table VIII.

It is evident that yeast B is slightly slower in activity than in the presence of sugar but thereafter is very fast. In the sugar it is by far the most active of the three yeasts in the series.

The data indicate that the method suggested can be used to determine the uniformity of any one type of yeast and to make comparisons between different brands. However, anyone using the pressure-meter method, either with or without sugar, to evaluate yeasts will have to familiarize himself with the correlation to be expected between results of the test and actual commercial bake shop practice.

Thus one hour of fermentation of the dough in the pressure meter at 30° C. corresponds to about the first hour of commercial sponge fermentation, depending on temperature at which the sponge was made and whether or not some salt was used. The percentage of yeast (3.0%), however, is not far different from that present in the commercial sponge if we consider 1.75% yeast, based on total flour, as an average amount used by the baker.

For obtaining information regarding the behavior of yeast in sponge fermentation a six-hour testing period is not necessary. From our experience all necessary information is obtained from the four-hour period. This time without sugar shows quite definitely differences in yeast activity that are valuable in making comparisons of different types of yeast for sponge fermentation.

TABLE IX  
GAS PRODUCTION FOR FOUR YEASTS WITH NO SUGAR AND WITH  
0.3 GRAM SUGAR

Time	No Sugar				Sugar			
	A	B	C	D	A	B	C	D
hrs.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
1	99	75	85	82	111	117	109	128
2	151	132	137	138	142	134	119	125
3	134	125	140	147	137	122	117	123
4	48	62	42	54	111	110	106	112
5	28	33	34	38	91	92	79	81

We made experiments to determine what effect the rate of gas production has on the time of pan proof of a straight dough, using the A.A.C.C. baking test and with modifications to duplicate commercial straight doughs. The yeasts used gave the pressure-meter readings, with and without sugar, shown in Table IX.

When the A.A.C.C. test doughs were made, 15 grams of the dough



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Time	No Sugar				Sugar			
	A	B	C	D	A	B	C	D
hrs.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
1	99	75	85	82	111	117	109	128
2	151	132	137	138	142	134	119	125
3	134	132	140	147	137	122	117	123
4	48	62	42	54	111	110	106	112
5	28	33	34	38	91	92	79	81

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When the A.A.C.C. test doughs were made, 15 grams of the dough

were placed in the pressure meter and readings taken every hour. The data thus obtained are given in Table X and graphically in Figures 3 and 4, in which is shown the gassing rate at the time the dough was put in the oven.

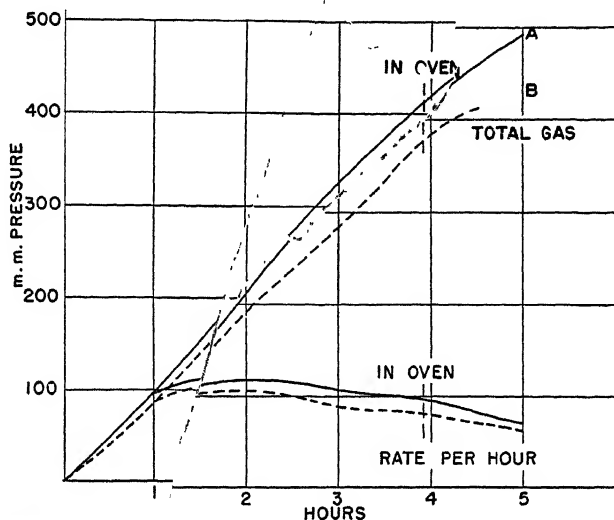


Fig. 3. Gas production with yeasts A and B, with A.A.C.C. baking-test doughs.

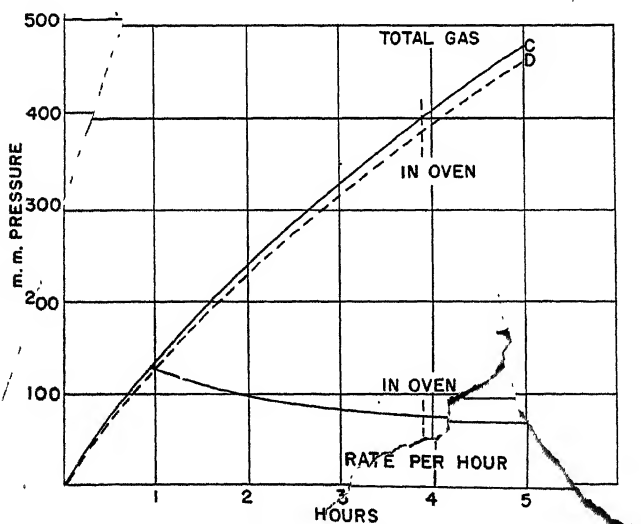


Fig. 4. Gas production with yeasts C and D, with A.A.C.C. baking-test doughs.

TABLE X  
GAS PRODUCED FROM 15 GRAMS A.A.C.C. TEST DOUGH<sup>1</sup>

Time	Yeasts			
	A	B	C	D
<i>hrs.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
1	96	87	133	125
2	117	106	105	106
3	109	88	94	91
4	97	79	73	72
5	72	65	72	77
Proofing time ( <i>min.</i> )	55	72	55	55

<sup>1</sup> Time of running and the flours were not the same for yeasts A and B as for C and D.

There was a difference in the activity of the doughs as they fermented, which was reflected in the height to which they rose; but only the proofing time, which is the most critical stage from the practical standpoint, was recorded. This is given in Table X. The data in this table show that the differences between the yeasts found by the pressure-meter readings are of significance. Thus yeast B is slower than yeast A in the pressure-meter reading with sugar and is also slower in liberating gas in the A.A.C.C. test and slower in pan proof. On the other hand, yeasts C and D are similar in both the pressure-meter and in the test dough, including proofing time. This verifies the thought expressed by many that any standardization of the A.A.C.C. baking test must take into consideration the activity of different types of yeast.

These results show that for straight doughs baked by the A.A.C.C. procedure the pressure-meter readings obtained in the manner suggested give a very definite valuation of the gas-production capacity of a yeast. Additional tests made with commercial straight doughs showed similar results, although in these tests the slower activity of the yeast due to higher concentration of salt and smaller amounts of yeast did not reveal as large differences in proofing time and in some cases completely eliminated the slight difference in proofing time which was noted between yeasts with the same flour when baked by the A.A.C.C. procedure.

### Sponge Baking Tests

Our results  
commercial  
the pro-

very sponge baking tests designed to imitate  
that the gas production in the sponge  
is as found by the pressure-meter  
from the similar composition of the  
dough used in the pressure meter. We

did not attempt to correlate pressure-meter method with volume of the sponge as this was affected by too many factors and it does not appear to be of much value.

In our experiments we prepared a dough baked in following composition:

Flour	60.0 grams
Water	6.0 grams
Yeast	2.0 grams
Yeast food	0.25 gram
Salt	0.25 gram

In one series of experiments the temperature of the water bath was started at 76° F. and the temperature of bread raised two degrees per hour until at the end of 5 hours the temperature was 86° F. This is approximately the rate at which commercial sponges increase in temperature. The pressure measured at any hour also measures the volume increase due to effect of increase in temperature, but this obviously is constant and was disregarded.

The dough was made by adding 7 g. of flour, 0.5 g. sugar, and

The results are shown graphically in Figure 5. Data are

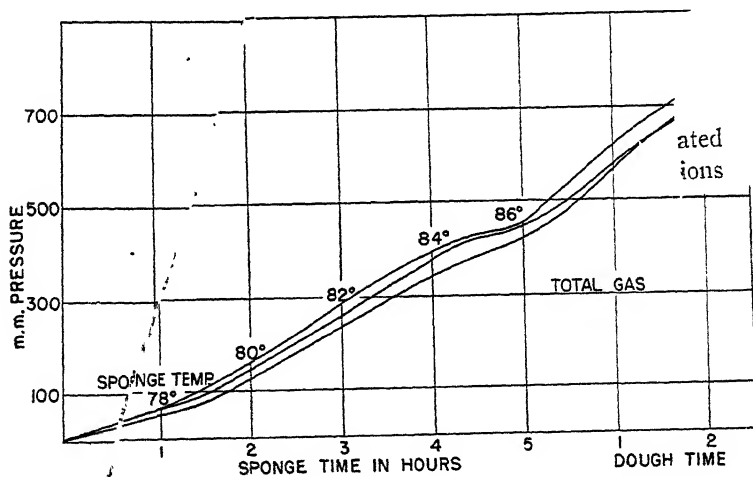


Fig 5. Gas production in sponge doughs with progressively increasing temperatures.

in Table XI. It is evident that the three yeasts show the same activity in the sponge as in the pressure-meter method, the results of which are shown in Table XII.

Yeast A is most active and produces undecarboxylated gas by both the regular pressure-meter method and to compare conditions set up in the test. Yeasts A and B had appeared to be the natural sucrose of the flour and the maltose formed during the cooler stages of

mentation before which was producing more gas per hour at the end of the fifth hour. No doubt if allowed to continue would have produced as much as A and B. The yeasts exhibited the same behavior in this stage. Yeast A is most active. In the tests made yeast A was more active than B at this stage.

## GAS PROD.

## EXE XI

106

88

79

65

## PONGE-DOUGH PROCESS

millimeters

		65					
Temp. sponge	Time (hrs.)	72			Rate per hour		
		A	B	C	A	B	C
Sponge							
76	1	42	54	45	68	54	45
80	2	164	141	124	96	87	79
82	3	289	259	228	125	118	104
84	4	398	382	339	109	123	111
86	5	447	434	417	49	52	78
Dough							
his tat	1	168	140	148	168	140	148
pressure.	2	298	261	284	130	121	136

TABLE XII

## COMPARISON OF YEASTS

Pressure-Meter Readings with 0.3 g. Sugar

Yeasts	Hours						Total
	1	2	3	4	5	6	
A	129	132	108	104	89	88	650
B	120	137	116	90	79	68	610
C	110	131	107	95	87	83	613

We found that the pressure-meter evaluation of the activity of the yeasts in presence of sugar was related to the time required to proof the dough to a definite height. Final loaf volumes, however, are the result of the many factors which influence the maturing and gas-retention properties of a dough and hence did not always correlate with pan-proofing time. This is the reason we have found it desirable to segregate gas-production activity from other properties as much as possible.

We also did the dough-expansion test, which is called by yeast comparison, to say definitely that the pressure-meter method suggested more valuable information. We do

wish to point out that the dough-expansion method is obviously designed for testing yeasts used in straight doughs and it does not give the information desired for yeasts used in sponge doughs by which, as claimed by most authorities, 90% of the bread baked in this country is made. The dough-expansion method does not take into consideration the different behavior of various types of flour. Any method which separates gassing strength of yeast from other properties and uses flour only as a substrate in which to test the yeast, is desirable.

Various types of flour differ so much in their reaction to proteolytic enzymes and acidity that it is not possible to judge the relative gassing power of yeast from the loaf volume of bread made by any one formula using various brands of yeasts.

We believe the suggested pressure-meter method with and without added sugar gives much valuable information regarding the ability of yeast to produce carbon dioxide from a dough, but that it must be supplemented by baking tests in order to determine the effect of other properties of the yeast on the maturing of the dough and character of the bread. Our experience has been that the pressure-meter readings are a very valuable guide in helping evaluate yeast.

### Summary

The Sandstedt-Blish pressure meter is an excellent piece of equipment for conveniently determining the gas-producing strength of doughs with or without added sugar. It can be used to determine the uniformity of any one type of yeast or to make comparisons between various types. The results obtained are a valuable aid in evaluating yeasts but should be supplemented by baking tests to determine the effect of other characteristics of the yeast on maturing of the dough and on bread quality.

Additional evidence is shown in support of Sandstedt and Blish (1934) that yeast variability is usually of very little significance in determining the gassing power of a flour, provided of course that fresh yeast is used.

Data are shown which indicate the necessity of knowing the source and type of yeast when making studies of rate of gas production for flour. Yeasts cannot be used interchangeably for this purpose; previous testings on a known or standard flour have shown that similar results are obtained only with flour

The effect of type and activity of yeast is an important factor in the A.A.C.C. baking test and any standardized method to determine certain specifications designed to compare two experimental yeasts.



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## A RAPID METHOD FOR THE DETERMINATION OF WHEAT AND FLOUR PIGMENTS<sup>1</sup>

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(Read at the Annual Meeting, May 1938)

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meter and colorimetric methods have been described for estimating thought pigment content of cereal products which are based essentially upon ~~baking~~ "gasoline color value" test devised by Winton (1911). In general,

Yeasts

methods require an extended time of extraction, usually over night, are thus not suited to problems involved in mill control. Coleman and Christie (1926) utilized high-speed stirring to accelerate the extraction, and in this manner succeeded in reducing the time required to 30 minutes. Their method, however, necessitates the use of a separate stirring motor of the malted-milk-mixer type for each test and is therefore, from the standpoint of cost, not well adapted to carrying out a number of tests simultaneously.

In addition to the lengthy extraction time required by the older result of, difficulty was frequently encountered in matching the extracts retention against the potassium chromate standards. This objection pan-proofing by Geddes, Binnington, and Whiteside (1934), who em-segregate gas

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Laboratory, Board of Grain Commissioners for Canada.

ployed unfiltered mercury-arc radiation as an illuminant and developed a colorimetric method in which the carotene equivalents corresponding to selected concentrations of potassium chromate were employed directly. This method is described in "Cereal Laboratory Methods" as an alternative to the spectrophotometric procedure for the determination of carotenoid pigments. While more accurate, this method is no more rapid than the conventional gasoline color value test.

In these prior studies, the investigators employed some form of petroleum hydrocarbon as a solvent, either alone or in admixture with absolute ethyl alcohol. Binnington, Sibbitt, and Geddes (1938) pointed out the deficiencies of such solvents and carried out an extensive survey of a number of commercially available organic compounds which resulted in the selection of water-saturated *n*-butyl alcohol as most nearly approaching the ideal. Experience gained with this solvent over a period of a year has confirmed its desirability and indicated the possibility of developing a rapid, simple, and accurate method involving the minimum of expenditure for special equipment. Such a method, if available, would appear to be of considerable practical value particularly in mill control. In the present paper a method which meets the requirements of rapidity and accuracy, together with simplicity and low equipment cost, is described.

### Experimental

In developing a rapid colorimetric method using water-saturated *n*-butyl alcohol as a solvent, it is necessary to determine the conditions under which maximum extraction can be secured in minimum time, develop a method of clarification which would preferably not involve the use of a centrifuge, and recompute the carotene equivalents of the potassium chromate standards for this solvent. For measuring the carotene content of the extracts the A. A. C. C. spectrophotometric method employing Bausch and Lomb instrument equipped with a scale reading directly in percentage transmittancy was used; a sample-to-solvent ratio of 20 g. to 100 c.c. was used in all cases. The transmittancy readings obtained in a 10 cm. cell were converted into the carotene equivalents by means of a table based upon the specific transmissive index (*K*) for carotene of 1.6632 in *n*-butyl alcohol—the value found by Binnington, Sibbitt, and Geddes (1938).

*Time of extraction.*—The results of a preliminary study with flour indicated that maximum extraction was obtained in 45 minutes without continued shaking; accordingly, experiments were undertaken to determine the shortest time required. For this purpose two experimentally milled unbleached and two commercially milled bleached flours were selected. The procedure followed consisted simply of adding the solvent

to the flour, shaking by hand to ensure thorough mixing, standing for the prescribed length of time, and shaking immediately before centrifuging. The results of duplicate tests are presented in Table I and show that extraction is complete in all cases at the expiration of 15 minutes. Within the range studied (0.92 to 3.05 p.p.m.) the actual pigment content would appear to be without influence upon the results.

TABLE I  
EFFECT OF EXTRACTION TIME UPON THE QUANTITY OF PIGMENT  
REMOVED FROM HARD RED SPRING WHEAT FLOURS

Carotene p.p.m. (spectrophotometer)								
Time of extraction	Exp. milled unbleached flour <sup>1</sup>		Exp. milled unbleached flour <sup>2</sup>		Com. milled bleached (med. grade)		Com. milled bleached (high grade)	
Min.	a <sub>1</sub>	b <sub>1</sub>	a	b	a	b	a	b
5	2.97	2.96	1.91	1.92	1.81	1.83	0.92	0.92
15	3.05	3.04	2.06	2.05	1.90	1.87	0.93	0.93
30	3.06	3.05	2.06	2.06	1.87	1.87	0.93	0.93
60	3.05	3.06	2.07	2.06	1.88	1.88	0.93	0.93
16-18 hrs. (over night)	3.05	3.05	2.09	2.08	1.85	1.87	0.93	0.92

<sup>1</sup> Letters a and b represent duplicate tests.

TABLE II  
EFFECT OF EXTRACTION TIME AND TYPE OF GRIND UPON THE QUANTITY OF  
PIGMENT EXTRACTED FROM HARD RED SPRING AND DURUM WHEATS

Sample	Grind	Carotene p.p.m. (spectrophotometer)					Remarks
		Time of extraction					
		½ hr.	1 hr.	2 hrs.	4 hrs.	16-18 hrs. (over night)	
1 Hard	Wiley, ½ mm. screen	3.45 3.51	3.64 3.63	3.65 3.63	3.65 3.67	— —	Standing <sup>1</sup> Shaken <sup>2</sup>
1 Hard	Allis-Chalmers mill	3.45 3.45	3.65 3.61	3.66 3.63	3.64 3.66	— —	Standing <sup>1</sup> Shaken <sup>2</sup>
2 C. W. Durum	Hobart <sup>3</sup>	4.22 4.22	5.30 5.30	5.34 5.32	5.54 5.56	5.86) 5.84)	Duplicate tests
1 C.W. Garnet	Hobart <sup>3</sup>	4.62 4.64	5.30 5.30	5.50 5.48	5.54 5.58	5.72) 5.72)	Duplicate tests
1 Nor.	Hobart <sup>3</sup>	3.34 3.34	3.98 4.02	4.04 4.04	4.00 4.00	3.98) 4.00)	Duplicate tests

<sup>1</sup> Shaken by hand at commencement and end of test.

<sup>2</sup> Shaken mechanically throughout entire extraction period.

<sup>3</sup> Finest possible grind employing Hobart Model 6 burr mill (equipped with stationary burr No. 4317, No. 2 R.N. Sta. MCH No. 6 Ex P.G. and rotary burr No. 4318, No. 2 P.H. Rot. MCH No. 6 Ex P.G.) with a setting of from 5.0 to 5.5.

TABLE III  
EFFECT OF EXTRACTION TIME AND TYPE OF GRIND UPON THE QUANTITY  
OF PIGMENT EXTRACTED FROM DURUM WHEAT SEMOLINA

Sample	Grind	Carotene p.p.m. (spectrophotometer)					Remarks
		Time of extraction					
		½ hr.	1 hr.	2 hrs.	4 hrs	16-18 hrs. (over night)	
Commercial No. 1	Wiley, ½ mm. screen	3.25 3.28	3.75 3.86	3.83 3.88	4.18 4.20	—	Standing <sup>1</sup> Shaken <sup>2</sup>
Commercial No. 1	Allis-Chalmers to unbolted flour	4.24 4.43	4.47 4.49	4.52 4.58	4.52 4.67	—	Standing <sup>1</sup> Shaken <sup>2</sup>
Commercial No. 1	Hobart <sup>3</sup>	—	—	—	—	3.88	Standing <sup>1</sup>
Commercial No. 1	Hobart <sup>3</sup> residue on 72 G.G.	—	—	—	—	3.70	Standing <sup>1</sup>
Commercial No. 1	Hobart <sup>3</sup> residue on 10 XX	—	—	—	—	4.08	Standing <sup>1</sup>
Commercial No. 1	Hobart <sup>3</sup> throughs 10 XX	—	4.62	—	—	4.62	Standing <sup>1</sup>

<sup>1</sup> Shaken by hand at commencement and end of test.

<sup>2</sup> Shaken mechanically throughout entire extraction period.

<sup>3</sup> Finest possible grind employing Hobart Model 6 burr mill (equipped with stationary burr No. 4317, No. 2 R.N. Sta. MCH No. 6 Ex P.G. and rotary burr No. 4318, No. 2 P.H. Rot. MCH No. 6 Ex P.G.) with a setting of from 5.0 to 5.5.

Studies were next conducted upon durum wheat semolinas and hard red spring and durum wheats, the results of which are detailed in Tables II and III. In the case of these materials, preliminary grinding is necessary and the selection of a suitable mill or grinding process represents the major difficulty in applying short-time extraction. In the instance of hard red spring wheat, a one-hour extraction appears to be adequate if the sample is either reduced to meal of flour-like fineness on an experimental mill or ground to pass a ½ mm. screen on the Wiley mill. When the Hobart grinder is employed, however, extraction is not complete in all cases even in four hours, although replicate tests at any given time are in excellent agreement. It will be noted that the Hobart grind, in the instance of the No. 1 Northern samples, is essentially constant at one-hour extraction, but this condition does not hold good for either the Garnet or durum wheats, which are of higher pigment content. Until a more satisfactory method of grinding is developed, it would appear desirable to utilize a longer extraction time for ground wheats or else determine the minimum time for the type of wheat under consideration. The results obtained with durum semolina are similar to those obtained with wheat; they emphasize the necessity of grinding to flour-like fineness if short extraction times are to be employed.

*Clarification.*—The production of a perfectly clear extract from flour suspensions has generally necessitated the use of a large-capacity high-speed centrifuge. Filtration through paper, alundum, or sintered

glass has been resorted to when such equipment was not available; in addition, the difficulty of securing clear extracts some adsorption of the pigment takes place when filter paper is used, as indicated by Ferrari and Bailey (1929).

TABLE IV  
COMPARISON OF FILTRATION VS. CENTRIFUGING AS A MEANS  
OF CLARIFYING FLOUR EXTRACTS

		Carotene p.p.m. (spectrophotometer)			
Original flour extract (centrifuged)		2.46			
Extract filtered through a No. 1 Whatman paper		2.44			
Extract filtered through a No. 2 Whatman paper		2.44			
Extract filtered through a No. 4 Whatman paper		2.45			
Sample No.		Carotene p.p.m. (spectrophotometer)			
		Centrifuged		Filtered (No. 1 Whatman)	
		<i>a</i> <sup>1</sup>	<i>b</i> <sup>1</sup>	<i>a</i> <sup>1</sup>	<i>b</i> <sup>1</sup>
1	Exp. milled flour (unbleached)	3.05	3.05	3.05	3.05
2	Exp. milled flour (unbleached)	2.06	2.08	2.09	2.08
3	Com. milled flour (bleached)	1.86	1.86	1.85	1.87
4	Com. milled flour (bleached)	0.92	0.93	0.93	0.92

<sup>1</sup> Letters *a* and *b* represent duplicate tests.

The use of water-saturated n-butyl alcohol has been found to flocculate flour to a marked extent, thus enabling brilliant extracts to be obtained by simple filtration through paper; in addition the presence of water in the solvent might be expected to reduce adsorption. Studies were made to investigate this point and the data presented in Table IV clearly indicate that adsorption does not take place to a measurable extent. Furthermore, the flocculating effect is so marked that a wide range of filter-paper grades may be used with equally satisfactory results. From general considerations, however, a medium-speed paper of fair density, such as a No. 1 Whatman, should be selected.

*Colorimetric estimation of pigment content.*—In the instance of a simple method, the pigment content of the extracts is most conveniently determined colorimetrically by matching against potassium-chromate standards as outlined in the method of Geddes, Binnington, and White-side (1934) and also Cereal Laboratory Methods, Section III, 196, employing unfiltered mercury-arc radiation as an illuminant. Recent developments in the lamp industry have made available an inexpensive mercury-vapor lamp of high intensity operating upon alternating current through the medium of an auto-transformer. A light source of this type<sup>4</sup> has been employed by the authors for some time, replacing the more expensive quartz mercury-arc with entirely satisfactory results.

<sup>4</sup> General Electric Co. Type H-2 250-watt T-9 medium-screw 120-volt mercury-vapor lamp and No. 58G43 transformer.

Actual matching of the extracts is preferably carried out by the "Standard Series" procedure outlined by the above authors but a colorimeter of the Duboscq type may be utilized. For a limited amount of testing, a simple rack and reflector will suffice, although a device of the type described by Whiteside, Edgar and Goulden (1934) will be found very convenient if a large number of determinations are to be conducted.

*Potassium-chromate standards.*—Because of the different specific transmissive index of carotene in n-butyl alcohol as compared with naphthal-alcohol, the standards described by Geddes, Binnington, and Whiteside (1934) cannot be employed without recalculation of their carotene equivalents or revision of their concentrations. The latter course appeared preferable in order to provide a series corresponding to even units of carotene. These revised values have been computed and are presented in Table V for a range of 0.2 to 4.0 p.p.m. of carotene.

TABLE V

POTASSIUM CHROMATE REQUIRED TO PRODUCE STANDARDS EQUIVALENT TO 0.2 TO 4.0 PARTS PER MILLION CAROTENE (IN INCREMENTS OF 0.2 P.P.M.) IN FLOUR<sup>1</sup>

Carotene value	0.5% potassium chromate per 1,000 c.c.	Carotene value	0.5% potassium chromate per 1,000 c.c.
<i>p.p.m.</i>	<i>c.c.</i>	<i>p.p.m.</i>	<i>c.c.</i>
0.2	0.78	2.2	8.53
0.4	1.55	2.4	9.30
0.6	2.33	2.6	10.08
0.8	3.10	2.8	10.85
1.0	3.88	3.0	11.63
1.2	4.65	3.2	12.43
1.4	5.43	3.4	13.18
1.6	6.20	3.6	13.95
1.8	6.98	3.8	14.73
2.0	7.75	4.0	15.50

<sup>1</sup> Based on a sample-to-solvent ratio of 20 g. flour to 100 c. c. of a solvent consisting of water-saturated n-butyl alcohol.

In order to test the accuracy of the revised standards, the pigment contents of a series of 39 flours were determined by both the spectrophotometric and colorimetric methods, single determinations only being made. The results presented in Table VI and shown graphically in Figure 1 indicate the essential accuracy of the standards. With reference to the absolute accuracy of the colorimetric method, it should be pointed out that in such a method of comparison, the onus of all the errors involved is thrown upon the colorimetric method, as the spectrophotometer values are taken as standard. Our experience is that the accuracy of the spectrophotometric method is in the same cal criterion 0.02 to 0.04 p.p.m.; the colorimetric method may thus be used for the mixer or the

satisfactory for all classes of work which do not involve the utmost precision.

TABLE VI

COMPARATIVE VALUES FOR CAROTENE CONCENTRATION IN FLOUR AS DETERMINED BY THE SPECTROPHOTOMETER AND THE REVISED POTASSIUM-CHROMATE STANDARDS EMPLOYING *n*-BUTYL ALCOHOL AS SOLVENT

Sample No.	Spectrophotometer (a)	Colorimeter (b)	Deviation (b) - (a)	Sample No.	Spectrophotometer (a)	Colorimeter (b)	Deviation (b) - (a)
	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>		<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
1	3.01	3.03	+ .02	21	1.28	1.25	- .03
2	2.78	2.77	- .01	22	1.04	1.05	+ .01
3	2.91	2.87	- .04	23	1.31	1.23	- .08
4	3.19	3.27	+ .08	24	0.87	0.95	+ .08
5	2.21	2.23	+ .02	25	1.06	1.10	+ .04
6	2.12	2.15	+ .03	26	0.80	0.93	+ .13
7	2.07	2.05	- .02	27	1.00	1.00	.00
8	2.19	2.17	- .02	28	0.70	0.75	+ .05
9	1.86	1.85	- .01	29	0.74	0.80	+ .06
10	2.34	2.30	- .04	30	2.93	2.95	+ .02
11	2.66	2.50	- .16	31	3.32	3.28	- .04
12	2.65	2.65	.00	32	2.36	2.35	- .01
13	2.66	2.70	+ .04	33	3.33	3.35	+ .02
14	2.61	2.55	- .06	34	3.69	3.75	+ .06
15	2.85	2.85	.00	35	3.32	3.37	+ .05
16	2.06	2.00	- .06	36	3.46	3.50	+ .04
17	2.39	2.50	+ .11	37	3.43	3.40	- .03
18	2.25	2.30	+ .05	38	3.83	3.87	+ .04
19	2.14	2.20	+ .06	39	3.93	3.95	+ .02
20	2.69	2.63	- .06				

Mean difference (b) - (a) = + 0.011 p.p.m.

*Rapid colorimetric method for carotenoid pigments in flour.*—The results of these studies, in which the colorimetric method described in Cereal Laboratory Methods has been modified to incorporate the rapid features made possible by the use of water-saturated *n*-butyl alcohol as solvent, are summarized below.

#### APPARATUS

- (1) *Comparator (Nessler) tubes.*—Clear glass tubes with fused-on plane parallel bottoms, 150 mm. high, 24 mm. diameter, wall thickness approximately 1.5 mm. Graduation marks to be etched at 40 and 80 mm. measured from the inside of the bottom surface. Comparator tubes may be obtained from Eck & Krebs, 131 West 24th St., New York, U. S. A.
- (2) *Color comparator.*—The color comparator comprises a quartz-mercury lamp (Alpine sun lamp or 250-watt mercury-vapour lamp similar to General Electric Company Type H-2) enclosed in a suitable ventilated housing, together with a rack for the comparator tubes and a magnesium oxide-coated reflector. The comparison rack may consist of a simple stand fitted with a clear glass shelf upon which the tubes may be arranged, the reflector inclined at a suitable angle beneath, or the more elaborate apparatus described in Cereal Chem. 11: 615. Readings must always be made by viewing vertically through the column of liquid.

mixing time and the fermentation time. This called for the baking of from 9 to 12 loaves from each sample of flour, which is impractical for routine testing. I am now presenting a modification of the variable method which I have found well adapted to the routine testing of flour samples of widely varying strengths.

I have retained the basic formula with the exception that the sugar is raised to 5%. Double the standard charge is weighed out. The absorption is varied with the protein content of the flour. This is in accord with the findings of Markley and Bailey (1938) in studies of dough formation in a recording dough mixer. A flour of 9% protein normally receives about 50% water, while one of 13% would get 62.5%. Occasionally a flour is found which requires more or less than the average for the protein content.

The mixing is carried out in the three-quart bowl of a Hobart mixer equipped with a paddle blade. The mixer is operated on slow speed for one-half minute to form the dough ball. When hard wheat flours are being mixed, the machine is then shifted to high speed and allowed to run until the doughs "clean up" or come free from the sides and start riding the paddle. The time required for this "clean-up" has been found to be quite highly correlated with the protein content. A low-protein hard-wheat flour will usually be well developed after one minute of high-speed mixing, while the high-gluten types may require  $2\frac{1}{2}$  minutes. Soft-wheat flours are mixed at medium speeds since they tend to become overdeveloped at high speeds before the dough is smooth.

After mixing the dough is placed in a two-pound butter crock. The standard bowl is too small for doughs from 200 g. of flour. The fermentation cabinet is held at 30° C. After 90 minutes of fermentation the dough is scaled into two 150-g. portions. One is returned to the cabinet and the other is panned. A standard high-form pan is used. It is proofed and baked according to the official method. The other portion is panned after 180 minutes of total fermentation. A second punch is given 20 minutes before panning. It is proofed and baked in the same manner as the first portion.

Variations are regularly introduced as supplements. Malt preparations, oxidizing agents, yeast foods, and improvers can be added to the basic formula. Other fermentation times and mixing times are occasionally useful. This test works well in determining the blending properties of flours.

It may be argued that it is not justifiable to compare loaves from different flours when the doughs have not been given exactly the same mixing time, but it appears more logical to use a biological criterion of mixing time, such as "clean-up" in the Hobart mixer or the



"sheeting" in the Swanson machine, rather than arbitrary units of time. In all biology it is very rare to find any process which runs exactly by the clock, and a dough is certainly a biological substance. Not only is the mixing time varied in this method of baking, but also the absorption is varied simultaneously with both the mixing time and the protein content. This is in accord with the findings of Markley and Bailey that there is a high inter-correlation between these three factors. This work indicates that both the absorption and the mixing time should be varied with the protein content if the doughs are to be mixed to the same stage of development. If doughs are not brought to the same stage of development it is unjustifiable to compare the finished loaves when flours are being evaluated. In the test method I find that an 11% protein flour at 57½% absorption is developed by one-minute of high-speed mixing to the same stage that a 13% flour is at 62½% absorption and two minutes of high-speed mixing.

The use of the uniform dough weight instead of uniform flour weight in each loaf is another of the long-debated points in test-baking technology. I recognize that the spread between flours in loaf volume tends to be narrowed when a uniform dough weight is used, but on the other hand it avoids the setting up of false differences between flours. The false differences, which are often found when the standard flour weight is used, are the cause of many of the discrepancies between the laboratory results and the findings when the flour is baked in the big shop.

This method of adapting the basic test to practical use appears to be advantageous in several ways. It is economical of flour, which is an advantage when experimentally milled flours are to be tested. Doughs are all given a uniform start by bringing them out of the mixer at uniform mobility and uniform stage of development. This makes the results quite comparable regardless of the strength of the flour. On this basic test many supplements can be superimposed.

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# THE COLLOIDAL BEHAVIOR OF FLOUR DOUGHS. V. COMPARISON OF THE INCREASE IN MOBILITY OF DOUGHS UPON EITHER PROLONGED MIXING OR FERMENTATION WITH THE EFFECTS OF VARIED MIXING TIMES UPON LOAF CHARACTERISTICS<sup>1</sup>

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American cereal chemists have been long accustomed to using the baking test as the final criterion of flour quality. Any mechanical device which is offered as a substitute for the baking test must yield values which show a high correlation with baking results if it is to be generally adopted in America (Geddes, Larmour, and Mangels, 1934). The Brabender-Hankoczy farinograph, from the claims made by its makers and from reports of European cereal chemists, appeared to have potentialities for the differentiation of wheat varieties, which is one of the major interests of the cereal laboratories of the Minnesota Agricultural Experiment Station.

The baking method used in these laboratories for variety testing was essentially Supplement D of the basic baking procedure of the American Association of Cereal Chemists. This supplement involved the variation of mixing time. With the spring-wheat flours mixing times of 2 and 5 minutes in the Hobart-Swanson mixer were employed. Enough malted wheat flour, approximately 1%, was used in order that no sample would suffer from diastase deficiency in the baking test. The farinograph, which records the changes in mobility of doughs during a prolonged mixing period somewhat similar to that given the doughs in the Hobart-Swanson instrument, possibly would give the same information as the mixing differential baking test.

In order to test this hypothesis a series of 23 spring-wheat patent flours milled in the Pillsbury 50-bbl. testing mill from the wheats grown in the Northwest Crop Improvement Association trials during the crop season of 1933 were baked by this differential mixing procedure and also were tested in the farinograph. The method with the farinograph was to mix doughs with 300 g. of the flours (13.5% moisture basis), 9 g. of yeast, 3 g. of salt, and  $7\frac{1}{2}$  g. of sugar with sufficient distilled water to yield doughs of a minimum mobility of  $550 \pm 25$  Brabender units at 30° C. The mixing period was prolonged to 40

<sup>1</sup> Paper No. 1610, Journal Series, Minnesota Agricultural Experiment Station.

minutes. The original titration curves (Brabender, 1932) were discarded and only smooth curves used in the study. After the mixing curve had been secured, a fermentation curve was made. The fermentation curve was identical with the mixing curve up to the time the dough reached the point of minimum mobility when the motor was cut off. The dough was allowed to rest for an hour in the mixer bowl at 30° and then the mixer was again put into motion. This second mixing was continued until the dough either recovered and passed through a new minimum or definitely did not recover. This was repeated hourly as long as desired. Examples of these two types of curves are given in Figure 1.

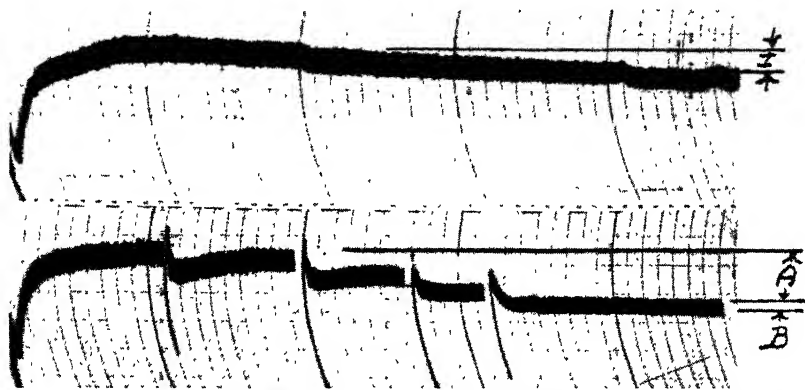


Fig. 1. Farinograms showing (above) an uninterrupted mixing curve; and (below) a curve of a dough mixed with the same flour to the point of minimum mobility, then allowed to ferment, and then remixed at intervals of one hour for a period of four hours

The presentation of all the individual scores for the many baking tests and farinograph charts would be of doubtful service and require much space, and so merely the means, standard deviations, and coefficients of variation are presented in Table I.

All but one of the samples represented commercially grown varieties, and the geographical distribution was fairly representative of that of the 1933 spring-wheat crop. Accordingly these 23 flours may be considered a representative sample of spring wheats and exhibit about as much variation as was encountered in that season. Of all the scores included in Table I only the absorption as determined by the farinograph was low in variability. There was as much differentiation in protein content as is usually met with in any one crop year. The mixing-stability score was calculated by dividing a composite quality score for the loaves from doughs mixed 5 minutes by the scores for the loaves from doughs mixed for 2 minutes. This quality score is based upon loaf volume, crumb grain, crumb texture, and external appearance of the loaf.

TABLE I  
STATISTICAL CONSTANTS FOR SPRING-WHEAT VARIETY PATENT FLOURS, 1933 CROP

		Mean	S.D.	C.V.
Baking Scores				
C	Loaf volume, 2 min. mixing <i>c.c.</i>	598.3	79.0	13.2
K	Loaf volume, 5 min. mixing <i>c.c.</i>	522.4	54.1	10.4
E	Mixing-stability score <sup>1</sup>	81.1	14.5	17.9
Farinograph Mixing Scores				
M	Absorption at 550 b.u. $\%$	58.8	2.4	4.1
I	Gain in mobility at 40 min. <i>b.u.</i> <sup>2</sup>	97.0	22.0	22.7
Farinograph Fermentation Scores				
A	Total gain in mobility in 4 hrs. <i>b.u.</i>	185.4	32.1	17.3
B	Recovery in mobility at 4 hrs. <i>b.u.</i>	19.5	17.8	91.2
L	Gain in mobility due to fermentation only (A-I) <i>b.u.</i>	88.5	25.3	28.6
Analytical Data				
J	Protein in flour $\%$	14.0	1.3	9.3
G	Diastatic activity (B and S) <i>r.u.</i> <sup>3</sup> n = 23	253.5	28.9	11.4

<sup>1</sup> Mixing stability score =  $\frac{.1 (\text{loaf vol.} - 200) + 2 \times \text{grain} + \text{texture} + \text{type for 5-min. mix}}{.1 (\text{loaf vol.} - 200) + 2 \times \text{grain} + \text{texture} + \text{type for 2-min. mix}}$ .

Grain, texture, and loaf type scored on scale of 0 to 10.

<sup>2</sup> *b.u.* = Brabender units, representing actual decrease in scale reading which involves an increase in mobility.

<sup>3</sup> *r.u.* = Rumsey units.

The coefficients of correlation for all possible combinations of these variables are given in Table II. The coefficients which exceed the 5% value for significance are printed in italics. From an inspection of this table it can be noted that the loaf volumes of the 2-minute-mix method were highly correlated with the protein content of the flour, but were

TABLE II  
COEFFICIENTS OF CORRELATION OF BAKING, FARINOGRAPH, AND ANALYTICAL SCORES

	K	E	M	I	A	B	L	J	G
C	<i>+.49</i>	<i>-.50</i>	<i>+.35</i>	<i>+.12</i>	<i>-.27</i>	<i>+.62</i>	<i>-.24</i>	<i>+.82</i>	<i>+.01</i>
K	—	<i>+.29</i>	<i>+.08</i>	<i>-.09</i>	<i>-.02</i>	<i>+.09</i>	<i>-.08</i>	<i>+.33</i>	<i>-.19</i>
E	—	—	<i>-.26</i>	<i>+.10</i>	<i>+.11</i>	<i>-.53</i>	<i>+.22</i>	<i>-.49</i>	<i>-.19</i>
M	—	—	—	<i>+.13</i>	<i>-.02</i>	<i>+.45</i>	<i>-.13</i>	<i>+.49</i>	<i>+.48</i>
I	—	—	—	—	<i>+.62</i>	<i>-.20</i>	<i>-.08</i>	<i>+.01</i>	<i>+.26</i>
A	—	—	—	—	—	<i>-.33</i>	<i>+.73</i>	<i>-.25</i>	<i>+.38</i>
B	—	—	—	—	—	—	<i>-.08</i>	<i>+.49</i>	<i>+.52</i>
L	—	—	—	—	—	—	—	<i>-.33</i>	<i>+.25</i>
J	—	—	—	—	—	—	—	—	<i>-.07</i>
	n = 23				5% point = .41				

independent of the diastatic activity. The volume of the 5-minute-mixed loaves was not significantly correlated with either the protein or the diastatic activity of the flour. The correlation between loaf volumes by the two methods, while significant, was not high. The mixing-stability score, which was calculated from the quality score for

5-minute-mixed loaf divided by quality score for 2-minute-mixed loaf, was negatively correlated with both 2-minute loaf volume and protein. Thus it appears that the relative damage to bread quality upon overmixing is the greatest in high-protein doughs.

This has been a frequent observation when using the mixing-time differential in the Hobart-Swanson mixer, although it is the reverse of what was commonly anticipated. The reason may be that there is more smoothing and compacting of the thick protein envelope about the starch granules than of the thinner envelopes in doughs of lower-protein flour. The absorption as determined in the farinograph is only moderately correlated with the protein content of the flour. The coefficient of correlation  $r = +.49$  is low because of the small variability in water-absorbing power of the flours used in this study, the coefficient of variability being only 4.1% and the range 9%. The gain in mobility of the dough during mixing, which is the mobility reading at 40 minutes subtracted from the minimum reading, was correlated with neither the 5-minute loaf volume nor the mixing-stability score. Neither was it correlated with either protein content or diastatic activity. It appears to be independent of those factors which we normally associate with bread-quality damage.

One explanation for the lack of a correlation between the increase in mobility of a dough upon overmixing and the damage to the finished loaf resulting from overmixing lies in an observation frequently made by baking technicians that often a dough which is very soft and ductile at the time of stopping the mixer quickly becomes stiff and short upon resting. This property is probably a starch function since it was shown in the first paper of this series (Markley, 1937a) that starch-water pastes in the dough range of mobility are thixotropic, having a gel structure when quiescent and becoming a sol upon agitation. The uniformity of the starches from different lots of wheat with regard to mobility of stiff pastes has never been investigated; it is possible that there may be much variation in this property.

Another explanation for the lack of correlation between the increase in mobility upon overmixing in the farinograph with the damage to bread by the same process may lie in the limitations of the baking test. The two mixing times, 2 and 5 minutes, were arbitrarily chosen without regard for the differences in time of development for the individual flours. Markley (1937b) has shown that with short fermentation times the loaf quality increases as the mixing of the dough is increased up to the limits of handling ability, while with long fermentation times the loaf quality generally decreases as mixing time is increased, the exact conditions being functions of the quality of the individual flour being tested. It is probable that the arbitrary mixing times of 2 and

5 minutes used in this study had no constant relation to the physical condition of the various doughs studied. If the mixing for the baking tests had been carried out in the farinograph, using the time to the minimum mobility of the dough and a definite increase in mobility, there might have been some relation between the shape of the farinograph curves and the results of the Supplement D as based upon a biological rather than a timed mixing differential.

It is probable that both of these explanations enter into the picture. It would be presumptuous for us to condemn arbitrarily a testing procedure merely because it does not conform to our present baking technique, which though useful is yet far from being a complete and perfect test. It appears certain that the observations made by means of the farinograph, and probably all other similar recording dough mixers, give us information which is distinct from that given by Supplement D (variation of mixing time) of the A. A. C. C. basic baking procedure. This lack of agreement does not condemn as worthless such physical techniques; they give very valuable supplementary information which is of value in determining how a flour will behave in the machines of the shop. A single baking test gives us practically no precise information on this point.

The fermentation curves as illustrated in Figure 1 indicate the extent to which dough mobility changes during fermentation. This is of special value to the baker as it lets him know for example just when a dough can no longer be worked without damage. The measurement of the increase in mobility at 4 hours (A) is the difference between the minimum mobility and the mobility at the secondary minimum at 4 hours if such appears, or is the mobility at 2 minutes if the mobility continually increases without the temporary decrease. The amount of this secondary decrease in mobility or increase in viscosity (B) was measured from the inflection point to the secondary point of minimum mobility.

The gain in mobility during 4 hours of fermentation (A in the tables) was not correlated with the baking scores; but was correlated with the increase in mobility due to overmixing (I). The correlation of  $r = +.38$  for diastatic activity (G) and the fermentation increase (A) approached statistical significance, and possibly may reflect the action of the  $\alpha$ -amylase upon the starch. The correlation between the fermentation increase (A) and the mixing increase (I) may be questionable since there was much additional mechanical energy put into the system during the hourly workings. With this point in mind the values for mixing increase (I) were subtracted from those values from the fermentation curves (A), with the resulting increases (L) due solely to the effects of fermentation. The values for L were found to be

independent of those for I, which indicates that the changes in the mobility of doughs upon fermentation are caused by factors other than those responsible for the slackening during prolonged mixing.

The shape of the hourly mixing curves resemble the thixotropic starch curves presented in the first section of this report. The rising of the curve due to redevelopment of the gluten is foreign to the starch curves, but after long fermentation the shape is identical with the starch type. There is one important difference between the starch and the fermentation curves in that the starch curves tend to repeat indefinitely the same mobility values, while the flour doughs continually increase in mobility from one hour to the next. Probably any or all of the many causes for the increase in mobility of the overmixed doughs as discussed by Markley (1937b) may enter into the fermentation increase, though not in the same proportions as in overmixing. In this group may be included mechanical damage caused by the gas expansion, proteolytic action,  $\alpha$ -amylase action, and any other type of damage.

The amount of the secondary decrease in mobility in the fermentation curve may be of importance. It (B) was positively correlated with the protein content, the 2-minute loaf volume, and the absorption, and was negatively correlated with the mixing-stability score (E). This may be interpreted as implying that high-protein flour doughs have enough gluten so that after four hours of fermentation they can be partially rebuilt in mobility and that such flours will yield the best bread. This recovery may be an elastic effect, since its decay during fermentation follows the rate of decay of elasticity as shown by Bohn and Bailey (1937).

### Summary

Increase in mobility of doughs upon prolonged mixing was not significantly correlated with the decrease in bread scores resulting from similar treatment, nor with the increase in mobility due to prolonged fermentation in the instance of doughs prepared from a series of 23 spring-wheat flours of the 1933 crop.

Increase in mobility of a dough upon prolonged mixing may be significant in the practical handling of doughs prepared from the same flour in a commercial shop when these relations are sufficiently established for the mixer and other mechanical facilities of the shop in question.

Conventional baking tests frequently are not designed for the adequate determination of mixing effects.

Temporary partial reconstruction of the fermenting dough upon reworking is apparently related to the production of optimum bread.

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## THE COLLOIDAL BEHAVIOR OF FLOUR DOUGHS. VI. DOUGH FORMATION FROM FLOURS OF DIVERSE TYPES<sup>1</sup>

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In the earlier papers of this series, certain basic principles involved in the application of recording dough mixers to dough fermentation have been discussed (Markley, 1937, 1938; Markley and Bailey, 1938). It appeared highly desirable to test these findings upon an extensive series of flours of diverse types. To this end a series of 89 flours was collected from many sources during the spring of 1934. These 89 flours included a group of experimentally milled Canadian protein composite flours from the Dominion Grain Research Laboratory, a series of commercially milled spring wheat flours from variety trials, a typical collection of Southwestern hard winter wheat flours of all grades, and a wide assortment of soft wheat flours, including four typi-

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in.

Doughs were mixed from each of these flours in the farinograph, rigid conditions being prescribed and maintained. All doughs made from 300 g. of flour at 13.5% moisture. The basic baking formula of the A. A. C. C. was employed for this study, which included yeast, 2½% sugar, and 1% salt. Sufficient water was employed to yield doughs with minimum mobilities of  $550 \pm 25$  units. Dough temperatures were held between 29° and 30° C. Under these conditions the quantity of water used (corrected to the 13.5% flour moisture basis) may be considered to be a function of the viscosity or mobility of the freshly mixed doughs, and the time required for the various doughs to reach the point of minimum mobility becomes a significant characteristic.

While Swanson (1936) in the study of flours of diverse strengths referred to use the supercentrifuge method of determining the proper absorption, yet in the light of the findings in the earlier portion of this study (Markley, 1937a) such a method did not appear justified. If the supercentrifuge method had been used for the determination of absorption, then there would have been a wide variation in the minimum mobility of the dough likewise, as is evident from Swanson's graphs, and a corresponding wide variation from commercial practice. It would have been impossible, also, to obtain comparative data respecting the time required to properly mix doughs prepared from the different flours.

On the other hand if the same quantity of water had been used with all flours there would have been difficulty in selecting a single absorption which would have yielded doughs possessed of mobilities within the range of the farinograph. Moreover, such doughs would have varied in mobility above and below that which is customary in commercial baking practice.

The farinograph records were used, therefore, as the measure of absorption. After a preliminary or exploratory titration test of absorption, smooth dough-development curves were traced by the farinograph. These were carefully measured for mobility at the end of five-minute intervals, and at the minimum point as well. The time in minutes required to reach the minimum mobility was also recorded, and the width of the line was noted for each such measurement. Certain of the significant data from these measurements, and the crude protein content ( $N \times 5.7$ ) are recorded in Table I. These data were also included in the statistical analysis.

The relation between absorption and protein content is shown graphically in Figure 1. The coefficient of correlation for these two

facts for the entire group was found to be  $r_{AE} = +0.71$ . Among the subgroups, it rose to the highest level,  $r_{AE} = +0.99$ , in the instance of the Canadian flours and fell to the lowest level,  $r_{AE} = +0.48$ , in the case of the Northwestern hard spring wheat flours. It is of special interest to note the lack of differentiation between the hard and soft wheat flours. Where there was similar protein content of the two types there did not appear to be any difference in absorption. There

TABLE I  
PROTEIN CONTENT AND FARINOGRAPH SCORES OF SAMPLES USED IN DOUGH  
FORMATION STUDY

Variety or grade	Source	Pro- tein %,	Absorp- tion %,	Mini- mum mobility B.U.	Time to point of minimum mob. min.
<i>Canadian Protein Composite Series, 1935 Crop</i>					
Exp. St.	Canada	11.4	55.5	555	7.2
"	"	11.8	56.0	545	10.0
"	"	12.3	56.5	560	9.0
"	"	12.8	57.5	545	10.0
"	"	13.1	58.0	575	9.7
"	"	13.6	58.0	560	12.0
"	"	14.2	59.0	575	8.7
"	"	14.7	60.0	555	14.5
"	"	15.4	61.0	550	14.2
"	"	15.7	62.0	555	17.0
<i>Miscellaneous Spring Wheat Flours</i>					
Patent	Minnesota	12.1	56.3	560	9.0
"	"	13.3	60.0	560	10.2
Straight	"	15.4	61.0	565	12.2
1st Clear	"	13.5	61.0	545	13.5
<i>Experimentally Milled Spring Patents, 1934 Crop</i>					
Marquis <sup>1</sup>		14.5	61.0	535	15.5
Ceres <sup>1</sup>		15.0	65.0	590	13.2
Hope <sup>1</sup>		15.7	60.5	560	15.0
Reward <sup>1</sup>		15.9	62.5	550	14.0
Thatcher <sup>1</sup>		16.1	61.5	555	15.0
H-44 X Marquis <sup>1</sup>		14.4	62.0	570	15.2
No. 2315 <sup>1</sup>		16.0	62.0	560	14.0
Marquis <sup>2</sup>		10.1	57.5	545	5.0
Ceres <sup>2</sup>		11.3	61.0	560	8.0
Hope <sup>2</sup>		11.8	59.0	550	10.0
Thatcher <sup>2</sup>		11.8	59.5	560	10.7

<sup>1</sup> Blends of equal parts wheat from St. Paul, Waseca, and Crookston, Minn.

<sup>2</sup> Blends of equal parts wheat from Grand Rapids and Duluth, Minn.

TABLE I—*Continued*

Variety or grade	Source	Pro- tein %	Absorp- tion %	Mini- mum mobility B.U.	Time to point of minimum mob. min.
<i>N. W. C. I. A. Spring Wheat Patents</i>					
Marquis	Fessenden, N. D.	13.6	60.0	540	9.5
Ceres	" "	14.0	62.7	575	10.5
Reward	" "	14.4	60.0	535	15.0
Marquis	Leeds, N. D.	13.5	58.3	540	12.2
Ceres	" "	14.6	62.0	570	9.2
Reward	" "	14.6	61.3	540	12.7
Marquis	Fargo, N. D.	13.7	57.2	560	10.0
Ceres	" "	15.1	64.3	560	10.2
Reward	" "	15.2	57.3	565	14.0
Thatcher	" "	14.5	60.7	555	10.0
Marquis A	Benton, Mont.	12.3	56.7	540	11.5
Ceres A	" "	11.6	58.7	530	10.0
Thatcher A	" "	13.2	56.3	540	11.5
Supreme A	" "	12.7	55.3	545	9.5
Comet A	" "	12.3	54.3	575	8.7
Marquis	Crookston, Minn.	11.8	57.7	540	8.0
Thatcher	" "	13.4	57.3	565	9.0
Marquis	Montevideo, Minn.	15.1	59.3	570	9.5
Thatcher	" "	17.0	57.7	560	13.0
Marquis	Morris, Minn.	14.8	59.0	560	11.0
Thatcher	" "	16.0	62.0	570	11.2
Marquis	Waseca, Minn.	13.3	56.0	550	8.0
Thatcher	" "	14.6	57.0	550	10.0

*Commercially Milled Hard Winter Flours, 1933 Crop*

Patent	Kansas	10.8	61.5	535	8.5
Clear	"	12.9	63.0	550	9.5
Patent	"	11.3	62.0	550	7.7
1st Clear	"	13.0	63.0	560	9.2
2nd Clear	"	15.3	66.5	560	10.8
Patent	Missouri	10.1	56.0	540	7.6
Clear	"	11.1	57.0	530	8.7
Patent	"	11.7	57.0	535	7.0
Clear	"	13.1	60.0	555	8.7
Patent	Texas	10.9	58.0	535	9.7
Clear	"	12.8	58.0	560	9.7
Patent	"	11.5	58.0	570	7.7
Clear	"	13.5	61.5	570	11.7
Patent	Kansas	10.4	58.0	555	6.5
Clear	"	12.2	60.0	550	8.2
Patent	"	11.4	60.0	550	9.2
Clear	"	13.9	63.0	565	11.0
Patent	"	11.8	61.0	545	8.2
Clear	"	14.4	63.5	535	10.5
Straight	Missouri	10.1	56.0	570	6.7
Patent	Kansas	11.0	56.0	560	7.5

TABLE I—Continued

Variety or grade	Source	Pro- tein %	Absorp- tion %	Mini- mum mobility B.U.	Time to point of minimum mob. min.
<i>Commercially Milled Soft Wheat Flours</i>					
Short Patent	Indiana	7.7	48.5	520	2.0
" "	Illinois	7.6	50.2	535	2.0
" "	"	7.9	49.1	550	3.5
" "	"	8.5	50.0	540	3.7
" "	Utah	7.5	50.0	550	2.2
" "	Missouri	9.3	53.0	555	3.7
Long Patent	"	9.6	53.0	560	3.5
Clear Patent	"	10.3	54.5	520	6.0
Long Patent	Kentucky	9.2	52.0	550	4.5
Patent	"	9.2	53.5	555	7.2
Clear	"	10.4	57.5	535	7.7
Hd. W. Pat.	Washington	11.4	56.5	500	5.0
Hd. W. Clear	"	14.2	63.0	535	7.2
Club Pat.	"	7.2	48.0	535	1.2
Club Clear	"	8.7	53.0	550	2.2
Patent	Germany	8.4	50.0	530	3.0
Clear	"	9.2	51.5	575	3.2
Baker's Pat.	"	9.9	55.0	565	6.0
Baker's Clear	"	10.8	57.5	545	4.5

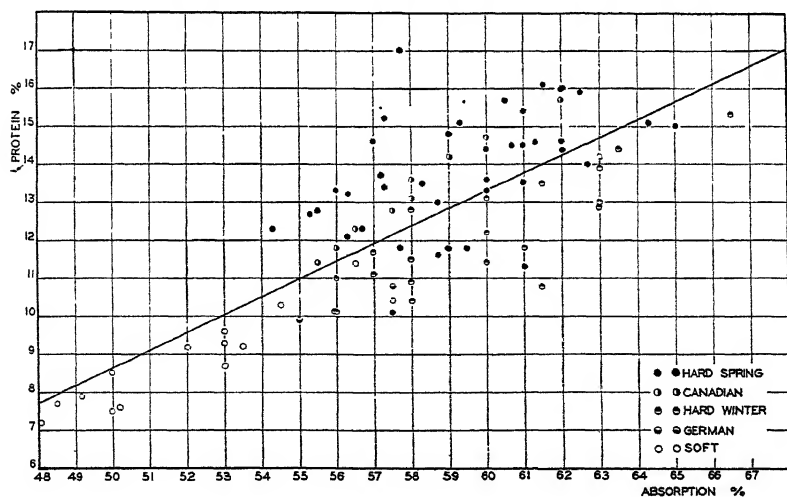


Fig. 1. Relation between the protein content of diverse flours and the absorption or the amount of water required to form doughs of a definite minimum mobility.

was some deviation from the fitted line, however. Those flours having absorptions lower than that predicted from their protein content, had, in this particular property of water-imbibing power, an inferior gluten quality, while those with higher absorptions had a superior gluten in at least that particular. This correlation suggests that instead of leaving the estimation of absorption for the basic baking test to the judgment of the operator, it might well be made a function of the protein content, with any necessary deviations from the average being considered as measures of deficiency or superiority in absorption. By this method the effect of protein quantity would be divorced from that of quality, which is a much needed addition to our test baking procedure.

TABLE II  
COEFFICIENTS OF CORRELATION BETWEEN FACTORS IN DOUGH FORMATION

	<i>AB</i>	<i>CD</i>	<i>AE</i>	<i>BE</i>	Number of samples	5% pt.	1% pt.
Entire series	+ .71	-.82	+ .77	+ .88	89	.24	.27
Soft wheat flours	+ .78	-.78	+ .96	+ .75	19*	.53	.58
Hard winter flours	+ .67	-.67	+ .80	+ .80	21	.50	.55
Northwestern hard spring flours	+ .44	-.43	+ .48	+ .67	39	.38	.42
Canadian flours	+ .88	-.86	+ .99	+ .85	10	.72	.76

*A*—Absorption (water required per 100 g. flour at 13.5% moisture to yield dough having a minimum mobility of  $550 \pm 25$  units).

*B*—Time in minutes required to develop doughs to minimum mobility.

*C*—Flour concentration in dough—grams per 100 g. water.

*D*—Logarithm of time in minutes required to develop dough to minimum mobility.

*E*—Crude protein content of flour ( $N \times 5.7$ ).

The time required to bring the doughs to their minimum mobility of 550 B.U. was found to be highly correlated with the protein content of the flour; the coefficient being  $r_{BE} = + 0.88$ , which is a very good agreement. The scatter diagram with the fitted line *As* shown in Figure 2. This is in close agreement with the findings reported earlier (Markley, 1938) upon the flour-starch-water doughs. On the basis of this study it is proposed that a more logical mixing treatment for the basic baking test than the present one of an arbitrarily fixed time would be a sliding scale of mixing times based upon protein content as derived from the average results of a great many flours. From the estimate of Bohn and Bailey (1936) that five minutes of mixing in the farinograph is approximately equal to one minute in the Hobart-Swanson it would appear that flours of 10% protein would be mixed to about their optimum by the basic baking procedure, while flours of lower protein content would be overmixed and the high-protein

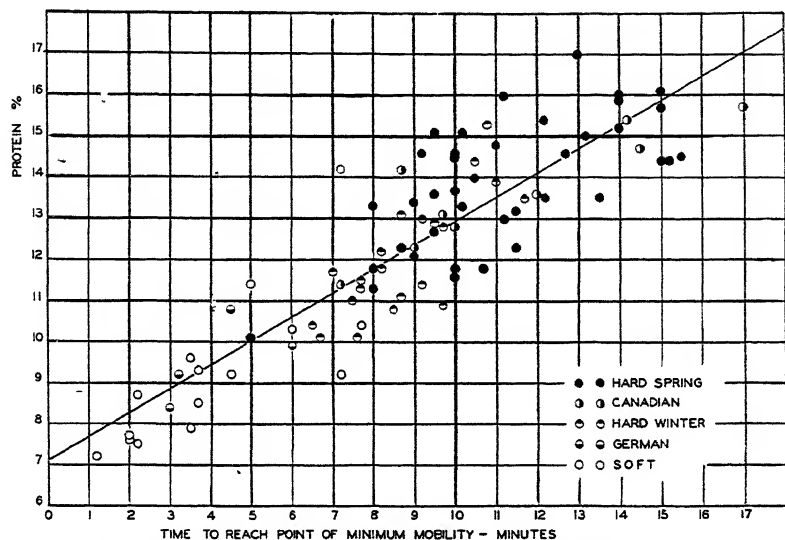


Fig. 2. Relation between the protein content of diverse flours and the time required to develop these flours into doughs of definite minimum mobility.

flours would be seriously undermixed. The soundness of the philosophy underlying the fixed mixing time is very questionable.

In Figure 3 are shown the relations between absorption and time required to bring the doughs to the constant minimum mobility in the farinograph for the different classes of flours. For the entire series the coefficient of correlation is  $r_{AB} = +0.71$ , and the individual classes range from  $r_{AB} = +0.88$  down to  $r_{AB} = +0.44$ . On the whole this relation appears to be curvilinear, so the values were recalculated as in section II (Markley and Bailey, 1938) to the basis of grams of flour per 100 grams of water and the log of the time. This

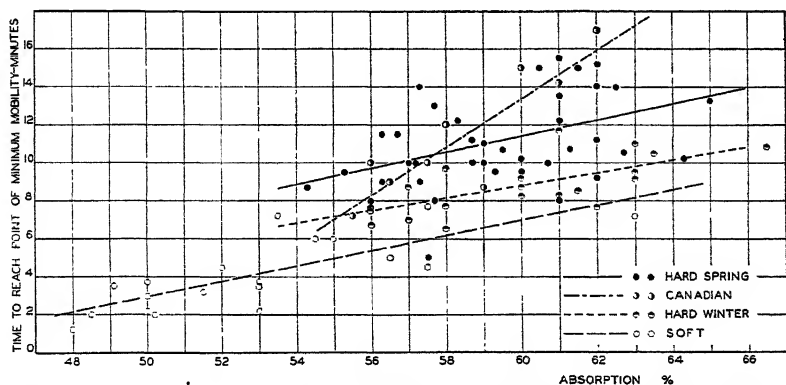


Fig. 3. Relation between the absorption of diverse flours and the time required to develop them into doughs of definite minimum mobility.

calculation straightened out the line, as may be seen in Figure 4. The coefficient of correlation for the series by this procedure was raised to  $r_{CD} = -0.82$ . There is good agreement with the earlier findings upon a few flours.

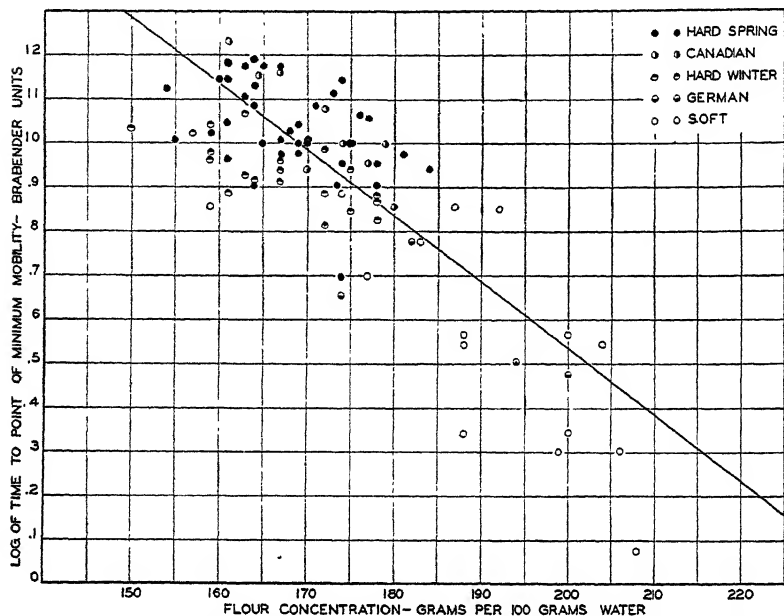


Fig. 4. Relation between the concentration of flour in doughs of definite minimum mobility with the log. of the time required to develop the dough.

The discovery of the close interlocking of protein content with both absorption and development time for doughs made from such a diverse group of flours as those used in this study opens a new approach towards standardizing the baking test. Thus it would be desirable to measure this relationship for a great many more flours from several crop seasons, and from the resulting mass of data secure accurate mean absorptions and mixing times for each protein level, using protein classes involving a range not greater than one percent. Then when a flour is found that deviates from this average in absorption and mixing time (either or both), as would many of the 1936 crop flours, it would be possible to report both an absolute value and a quality value for each factor.

### Summary

Dough formation in an extensive and diverse series of flours was studied. Protein content was found to be highly correlated with absorption and with dough-development time. Absorption was highly

correlated with development time. A scientific basis for a standardization of certain details of the baking test is suggested.

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### A STUDY OF GLUTEN PROTEIN FRACTIONATION FROM SODIUM SALICYLATE SOLUTION. PART IV. EFFECT OF PROTEOLYTIC ENZYMES, AS INFLUENCED BY CLASS OF WHEAT

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It is evident from a study of the literature that there is a diversity of opinion among cereal chemists respecting gluten quality differences. If gluten quality does vary, it would be logical to expect that differences would be found in the chemical characteristics of glutens washed from flours of different baking quality. Such differences were apparently discovered by Harris (1937, 1938) in the quantity of gluten protein removed from sodium salicylate dispersion of washed gluten by  $MgSO_4$ . A significant relationship between quantity of protein removed and loaf volume was found in two series of flours of 30 samples each. One series consisted of durum wheat, while the other was made up of hard red spring wheat. No significant relationship between crude-flour protein content and loaf volume was demonstrated. The standard basic method was used to determine baking quality.

This technique of gluten protein dispersion and fractionation by successive suitable additions of  $MgSO_4$  to sodium salicylate dispersions was applied by Harris (1938a, 1938b) to measure the effect of various proteolytic enzymes upon the glutens washed from doughs containing such enzymes. It was found that proteolytic enzymes did affect both rate of gluten dispersion and the relative distribution of the quantity of protein contained in three fractions removed from these



dispersions. One sample of hard red spring wheat flour was used as experimental material in this investigation.

Rupp and Bailey (1937) presented evidence to the effect that gluten from different types of flour is affected to different degrees by the same proteolytic treatment. These workers found that the rate of decrease in the development work (D.W.) of flours, as measured by the farinograph, varied with the quality of the flour used where the same proteolytic treatment was employed for each flour.

In view of these investigations and the results obtained therefrom, it was deemed advisable to apply fractionation methods to gluteins washed from doughs made up from samples of flours which had been milled from different classes of wheat and subjected to varying degrees of proteolytic treatment. From the results obtained from such a study, it would be possible to ascertain whether the effect of proteolytic enzyme treatments on the various classes of wheat flours would be reflected in the comparative protein distribution of their gluten fractions.

#### Experimental Material and Methods

Flours milled from four classes of wheat were used in this study. These wheats represented the hard red spring, hard red winter, soft red winter, and durum classes, and included two commercial and two experimentally milled flours. The papain preparation used in this work was prepared by Merck and Company and had been recently purchased. The pancreatin preparation had likewise been obtained from the same company. The yeast water used as a flour protease activator was prepared according to the method outlined by Jørgenson (1936) and was freshly made immediately before using. Papain was used because of its similarity to flour protease, and pancreatin was included to obtain data from a protease of animal origin.

The papain and pancreatin were dispersed in distilled water at suitable concentrations shortly before incorporation in the dough. After thorough shaking the required quantity of distilled water plus enzyme was added to the water necessary for correct absorption and then incorporated in the dough by a two-minute mix in the Hobart-Swanson. The usual quantities of yeast, sugar, and salt were employed.

The gluten was washed from the doughs immediately after mixing was completed, using a wash solution of pH 6.6 which contained 0.1% sodium phosphate. After the washed gluten had stood a short time under the sodium phosphate solution, 9 grams of wet crude gluten were weighed out, cut into small pieces, and placed in 150 c.c. of 10% sodium salicylate. After standing for several days, with frequent

shaking, the dispersions were centrifuged. The supernatant liquid was now decanted from the residual material in the centrifuge tubes. The dispersed gluten protein was then fractionated according to the procedure outlined by Harris (1938b), employing successive additions of 1.5, 3, and 10 c.c. of concentrated  $\text{MgSO}_4$  solution per 25 c.c. of dispersion. The volume of  $\text{MgSO}_4$  solution added to bring down fraction 2 was changed from 4 to 3 c.c. to leave a larger quantity of protein to be recovered in the third fraction.

The moisture determinations were run on 5 grams of wet crude gluten. Heating at approximately  $105^\circ \text{C.}$  was continued for 48 hours in a Freas drying oven.

### Discussion

The wheat and flour description, crude flour protein, flour ash, and the loaf volumes obtained by the standard basic and malt-phosphate-bromate baking methods are shown in Table I. Substantial differences in protein and gluten content and loaf volume are evident between the hard and the soft wheat samples. The soft winter wheat flour had a low ash content, while the durum straight flour was highest in this constituent. There was no great difference in loaf volume between the two hard wheats despite the higher protein content of the hard red spring. The durum, which is not classed as a bread wheat, yielded relatively low loaf volumes when protein and gluten content are taken into account. Both the spring wheats had exceptionally high dry gluten content in view of their percentages of crude flour protein.

TABLE I  
DESCRIPTION AND COMPARATIVE DATA FOR THE FLOURS USED IN THIS STUDY

Sample No.	Description	13.5% moisture basis				Loaf volume	
		Ash	Crude protein $\text{N} \times 5.7$	Wet crude gluten	Dry gluten	Stand- ard basic	Malt phos- phate bro- mate
38-1-56	Hard red spring wheat com- mercially milled	%	%	%	%	c.c.	c.c.
37-11-32	Hard red winter wheat ex- perimentally milled	0.44	13.2	46.1	14.5	577	688
38-4-8	Soft red winter wheat com- mercially milled	0.50	12.5	39.4	12.7	591	636
37-12-26	Mindum durum wheat ex- perimentally milled	0.28	7.5	20.4	7.1	379	349
		0.87	13.3	44.5	14.2	480	467

In Table II are shown the data obtained from the gluten washed from doughs treated with varying concentrations of papain, as well as doughs without enzymic treatment. No definite trends were established in regard to the gluten data, as marked differences must be found between the gluten contents from various doughs before such differences can be judged significant because of the lack of precision

TABLE II

GLUTEN-MOISTURE AND DRY-GLUTEN CONTENT OF FLOUR AND PROTEIN DISTRIBUTION EXPRESSED AS PERCENT OF TOTAL SOLUBLE PROTEIN IN DISPERSED GLUTENS WASHED FROM DOUGHS TREATED WITH VARIOUS CONCENTRATIONS OF PAPAIN

Flour No.	Treatment	Wet gluten moisture	Dry gluten <sup>1</sup>	Gluten solubility	Fraction			Total protein removed
					1	2	3	
		%	%	mg. per 100 c.c.	%	%	%	%
38-1-56 (Hard red spring)	Control, no yeast	67.9	15.0	1462	55.3	29.6	1.2	86.1
	Control, with yeast	67.3	14.5	1448	54.5	32.0	1.6	88.1
	Papain 0.001%	67.6	14.2	1530	46.8	39.8	3.0	89.6
	Papain 0.002%	68.6	14.0	1546	43.8	39.4	3.6	86.8
	Papain 0.004%	68.1	13.8	1502	26.0	52.1	9.2	87.3
	Papain 0.010%	68.1	14.2	1399	19.6	49.9	15.1	84.6
37-11-32 (Hard winter)	Control, no yeast	70.4	12.7	1271	47.8	34.3	5.0	87.1
	Control, with yeast	71.3	13.5	1237	47.4	38.2	2.7	88.3
	Papain 0.001%	71.1	13.8	1294	38.6	40.0	4.2	82.8
	Papain 0.002%	70.4	13.4	1260	30.0	49.9	9.2	89.1
	Papain 0.004%	71.5	14.1	1151	21.8	50.6	14.8	87.2
	Papain 0.010%	69.3	12.6	1420	12.1	43.7	21.7	77.5
38-4-8 (Soft winter)	Control, no yeast	65.1	7.1	1320	40.3	35.2	6.7	82.2
	Control, with yeast	68.9	7.1	1640	45.8	35.1	5.1	86.0
	Papain 0.001%	70.6	7.4	1334	40.2	36.2	8.4	84.8
	Papain 0.002%	67.4	7.8	1431	26.0	44.3	11.9	82.2
	Papain 0.004%	67.5	8.3	1391	23.3	42.6	15.1	81.0
	Papain 0.010%	68.6	6.8	1362	14.5	33.2	26.9	74.6
37-12-26 (Mildum durum)	Control, no yeast	68.1	14.2	1351	37.3	40.7	8.7	86.7
	Control, with yeast	66.9	13.6	1283	42.5	43.3	2.8	88.6
	Papain 0.001%	68.6	13.5	1357	31.8	43.5	10.4	85.7
	Papain 0.002%	66.9	13.6	1448	28.5	45.6	11.3	85.4
	Papain 0.004%	68.2	13.3	1388	35.8	41.7	7.9	85.4
	Papain 0.010%	66.2	8.8	1428	18.2	43.9	17.3	79.4

<sup>1</sup> Calculated to basis of 13.5% flour moisture.

in methods of determining gluten. The low results obtained in the instance of the 0.01% papain treatment of the durum dough was doubtless due to the loss of gluten material during the washing, as this particular dough was difficult to handle and wash. No general trend in the dry-gluten results appeared to be established with papain increments.

Upon examining the first fraction obtained by this method it is seen that the quantity of protein decreases in each class of wheat for increasing concentration of papain. It is also evident that the hard red spring wheat flour dough contained more protein in this fraction following substantial papain treatment than did the hard red winter or soft red winter. The durum results appeared to coincide very closely with the spring-wheat figures at the highest papain dosage employed, but did not resemble the values obtained for the other wheats at lower papain concentrations. The second fraction is increased in general by papain. This fraction varies from 49.9% of control for the hard red spring through 43.7% for the hard winter to 33.2% for the soft winter when 0.01% of papain was used. The durum is practically identical with the hard winter. There thus appears to be a slight trend toward decrease in quantity of protein removed in fraction 2 in going from hard red spring to soft red winter in this concentration of papain in this study. The other papain treatments are not very consistent.

The third fraction appeared to be definitely increased by papain treatment for all the four wheat flours, but this increase varied greatly among the samples. The hard red spring was the lowest, showing a value of 15.1% of control for the highest papain treatment of 0.01%, with the hard winter yielding 21.7% and the soft winter 26.9%, respectively. Fraction 3 accordingly showed a tendency to vary with flour protein and malt-phosphate-bromate loaf volume upon papain treatment in these different classes of wheat. Durum was intermediate between the hard spring and hard winter wheats. The same tendency is also seen when the control-dough results are examined. There is no definite evidence of an increase in the gluten protein fractionated. The solubility of gluten protein in 10% sodium salicylate does not appear to increase with the severity of papain treatment.

In Table III the results obtained by the three concentrations of pancreatin used are shown. An interesting situation is revealed when the quantity of protein removed in fraction 1 is examined. While a general decrease of protein removed in this fraction is shown with an increase in severity of treatment, the degree of decrease from the values yielded by the untreated doughs varies greatly from one wheat class to another, being least in the instance of the durum and greatest for the soft winter wheat. The quantity of protein found in the first fraction following treatment of 0.04% pancreatin ranged from 43.8% of control in the case of the hard red spring dough, to 14.5% for the hard red winter. The durum was second highest and the soft red winter dough next to the lowest. No marked differences are shown in the case of fraction 2, but with the third fraction removed a striking

increase in the quantity of protein thrown out of solution is evident in passing from the hard red spring data through the durum and hard winter to soft winter wheat results. These results correspond with a marked decrease in fraction 1 for the winter wheats. A shift toward the smaller region of particle size appears to be caused by enzymic action, and this effect is more marked in the winter wheat dough gluten dispersions. Some reduction in the quantity of gluten protein fractionated is also visible in these instances.

TABLE III

GLUTEN-MOISTURE AND DRY-GLUTEN CONTENT OF FLOUR AND PROTEIN DISTRIBUTION EXPRESSED AS PERCENT OF TOTAL SOLUBLE PROTEIN IN DISPERSED GLUTENS WASHED FROM DOUGHS TREATED WITH VARIOUS CONCENTRATIONS OF PANCREATIN

Flour No.	Treatment	Wet gluten moisture	Dry gluten <sup>1</sup>	Gluten solubility	Fraction			Total protein removed
					1	2	3	
		%	%	mg. per 100 c.c.	%	%	%	%
38-1-56 (Hard red spring)	Control, no yeast	67.9	15.0	1462	55.3	29.6	1.2	86.1
	Control, with yeast	67.3	14.5	1448	54.5	32.0	1.6	88.1
	Pancreatin 0.01%	67.7	14.7	1411	50.1	34.6	1.8	86.5
	Pancreatin 0.02%	67.2	15.6	1342	48.7	34.5	2.0	85.2
	Pancreatin 0.04%	67.4	15.2	1371	43.8	37.2	2.6	83.6
37-11-32 (Hard winter)	Control, no yeast	70.4	12.7	1271	47.8	34.3	5.0	87.1
	Control, with yeast	71.3	13.5	1237	47.4	38.2	2.7	88.3
	Pancreatin 0.01%	72.7	13.1	1191	41.6	40.0	3.9	85.5
	Pancreatin 0.02%	76.7	10.0	1180	15.2	41.4	8.5	65.1
	Pancreatin 0.04%	69.8	13.3	1270	14.5	41.9	9.4	65.8
38-4-8 (Soft winter)	Control, no yeast	65.1	7.1	1320	40.3	35.2	6.7	82.2
	Control, with yeast	68.9	7.1	1640	45.8	35.1	5.1	86.0
	Pancreatin 0.01%	68.3	7.0	1390	52.1	47.2	9.2	108.5
	Pancreatin 0.02%	69.4	8.0	1330	31.9	48.7	11.7	92.3
	Pancreatin 0.04%	66.0	6.6	1340	26.4	40.1	16.7	83.2
37-12-26 (Mindum durum)	Control, no yeast	68.1	14.2	1351	37.3	40.7	8.7	86.7
	Control, with yeast	66.9	13.6	1283	42.5	43.3	2.8	88.6
	Pancreatin 0.01%	69.2	12.6	1294	32.9	46.4	3.2	82.5
	Pancreatin 0.02%	69.1	13.5	1311	39.0	42.1	4.9	86.0
	Pancreatin 0.04%	68.6	13.8	1271	35.7	44.5	6.3	86.5

<sup>1</sup> Calculated to basis of 13.5% flour moisture.

The results obtained when various concentrations of yeast water were incorporated in the doughs are shown in Table IV. The hard winter wheat dough glutes were the highest in gluten moisture. The gluten moisture tended to be raised by yeast water. The crude gluten content was also increased in the majority of cases. The gluten solubility was somewhat depressed by yeast water except in the treat-

ments involving the glutens washed from doughs mixed from durum wheat flours. The first fraction appears to be depressed by the activation of the native flour proteases in the instance of each wheat studied, but this initial effect is not generally increased by heavier doses of yeast water. Harris (1938b) found an increase in the quantity

TABLE IV  
GLUTEN-MOISTURE AND DRY-GLUTEN CONTENT OF FLOUR AND PROTEIN DISTRIBUTION EXPRESSED AS PERCENT OF TOTAL SOLUBLE PROTEIN IN DISPERSED GLUTENS WASHED FROM DOUGHS TREATED WITH VARIOUS CONCENTRATIONS OF YEAST WATER

Flour No.	Treatment	Wet gluten moisture	Dry gluten <sup>1</sup>	Gluten solubility	Fraction			Total protein removed
					1	2	3	
		%	%	mg. per 100 c.c.	%	%	%	%
38-1-56 (Hard red spring)	Control, no yeast	67.9	15.0	1462	55.3	29.6	1.2	86.1
	Control, with yeast	67.3	14.5	1448	54.5	32.0	1.6	88.1
	Yeast water 10 c.c.	71.7	14.8	1243	40.7	46.6	2.2	89.5
	Yeast water 20 c.c.	71.3	15.2	1248	35.8	47.7	2.4	85.9
	Yeast water 25 c.c.	71.7	15.2	1214	40.8	42.7	1.3	84.8
37-11-32 (Hard winter)	Control, no yeast	70.4	12.7	1271	47.8	34.3	5.0	87.1
	Control, with yeast	71.3	13.5	1237	47.4	38.2	2.7	88.3
	Yeast water 10 c.c.	72.4	14.3	1151	40.8	44.4	3.3	88.5
	Yeast water 20 c.c.	73.3	13.5	1106	40.6	45.7	3.7	90.0
	Yeast water 25 c.c.	72.8	14.1	1140	36.0	44.0	6.0	86.0
38-4-8 (Soft winter)	Control, no yeast	65.1	7.1	1320	40.3	35.2	6.7	82.2
	Control, with yeast	68.9	7.1	1640	45.8	35.1	5.1	86.0
	Yeast water 10 c.c.	69.2	7.3	1340	43.7	38.8	6.0	88.5
	Yeast water 20 c.c.	68.7	7.3	1351	38.3	41.2	7.5	87.0
	Yeast water 25 c.c.	57.0	10.7	1288	37.0	42.2	7.1	86.3
37-12-26 (Mindum durum)	Control, no yeast	68.1	14.2	1351	37.3	40.7	8.7	86.7
	Control, with yeast	66.9	13.6	1283	42.5	43.3	2.8	88.6
	Yeast water 10 c.c.	67.5	14.3	1322	34.7	45.0	8.5	88.2
	Yeast water 20 c.c.	67.4	8.8	1419	33.4	43.1	8.0	84.5
	Yeast water 25 c.c.	70.6	12.7	1328	35.4	43.6	8.4	87.4

<sup>1</sup> Calculated to basis of 13.5% flour moisture.

of protein removed as fraction 1 following treatment with yeast water when using hard red spring flour milled from the 1936 wheat crop as investigational material. It was therefore postulated in the instance of this research that activated flour protease appeared to exert a coagulating influence upon flour gluten protein, and in this way differed from papain. No increase in the depressive effect was noted in the present instance when larger quantities of the activating liquid were added, however, and in this respect the results agree with the former conclusions of the author. It is possible that increasing the con-

centration of activator does not increase the activating effect, and this explanation may account for the failure of fraction 1 to decrease with increasing severity of yeast-water treatment, rather than to differences in the action of flour protease and papain. It does not seem probable that these four flours would contain equal quantities of protease, and therefore the lack of decrease in fraction 1 with increase in severity of treatment cannot be attributed to a lack of the enzyme content necessary to further affect the gluten. The other two gluten protein fractions do not appear to be affected to any marked degree by yeast water although a slight tendency toward increase in quantity of protein removed in fraction 3 is seen when progressively comparing the results from the hard red spring wheat with the hard winter and soft winter wheats.

The general effect of proteolytic enzymes in decreasing the quantity of gluten protein in fraction 1 was shown by all four flours for the three enzymes employed. The hard red spring wheat flour gluten had the largest proportion of protein removed in fraction 1, but this fraction was reduced by proteolytic action to a greater extent in this instance than in the other wheat flour glutes. The soft winter wheat glutes were the least affected in the class of bread wheats, while the winter wheat was intermediate in this respect. The durum showed the smallest difference between the dough without yeast or enzyme and the dough containing the highest dosage of enzyme with yeast. These relationships were true for the papain and pancreatin doughs, but were not true when yeast water was used as an activating agent for the flour protease. When pancreatin was used the hard red spring wheat glutes were not affected as markedly as were the hard winter, while the durum was least affected. The change in the relative ranking of the spring wheat when treated by pancreatin instead of papain was probably due to some difference in the effect of the two enzymes upon the flour gluten. The highest dosage of pancreatin used did not have as much effect as the 0.01% treatment of papain, and the spring wheat gluten may have been more resistant to its action than were the hard winter wheat flour glutes. This explanation does not seem very probable, however, in view of the results yielded by the soft winter wheat gluten, which showed less effect with pancreatin than with papain.

The hard red winter wheat sample showed a tendency to have the smallest quantity of gluten protein removed as fraction 1 following the addition of proteolytic enzymes to the dough. The soft red winter wheat, on the other hand, had the largest proportion of protein removed in fraction 3 as compared with the other samples. There appeared to be a distinct trend toward an increase in the third fraction

in going through the data from hard red spring to hard red winter, finishing with the soft winter. The durum samples yielded results nearest the hard red spring wheat. This trend applied to the papain and pancreatin treatments, and was not apparent when the data obtained by the yeast-water treatments was examined.

Fraction 2 appeared to decrease in proportion in going from hard red spring to soft red winter when the papain treatments were considered, as shown in Table II, while the hard red winter was intermediate in this respect.

It would seem that there is a difference in the aggregation of the gluten protein complex among the various classes of wheat examined in this study. Fraction 1 tends to decrease from the hard red spring wheat doughs to the winter wheat, both in the instance of the doughs treated with proteolytic enzymes and the untreated doughs. Fraction 2 also tends to change in much the same manner. Fraction 3 is increased as one goes through the data from the spring wheats to the soft winter. This change is also apparent upon proteolytic treatment. These results, it seems to the authors, may possibly be explained by the existence of smaller gluten particles in the case of the winter wheat as compared with the spring wheat. It is, however, realized that too rigid generalizations cannot be made from results obtained from an examination of only one sample of each of the classes of wheat included in this research.

### Summary and Conclusions

Four flours milled from hard red spring, hard red winter, soft red winter, and durum wheat were analyzed for ash, total protein, and dry crude gluten. These flours were also baked by two methods: the basic standard with 5% of sucrose and the malt-phosphate-bromate, which included 0.3% of 60° Lintner malt, 0.1% of ammonium phosphate, and 0.001% of potassium bromate in addition to the basic ingredients.

These flours were mixed into doughs in the customary manner with the exception that appropriate concentrations of papain, pancreatin, and yeast water were incorporated in the dough mix in all except the control doughs. The gluten was washed from these doughs immediately after mixing, and the percentages of gluten moisture and dry crude gluten determined. A portion of the wet crude gluten was dispersed in 10% sodium salicylate solution and the final concentration of dispersed gluten protein determined. The dispersed protein was then fractionally precipitated by successive additions of  $MgSO_4$  solution and the quantity of protein determined in each fraction.

The results obtained showed that proteolytic enzymes affect the relative distribution of the protein fractions in different classes of wheat



flour in the same general manner. The first fraction was progressively reduced in quantity as the concentration of enzyme increased. This effect is then reflected in a decrease in the second fraction as the severity of the treatment is increased. The quantity of protein removed in fraction 3, on the other hand, is augmented by this treatment.

The effect of papain and pancreatin upon the proportion of gluten protein contained in fraction 1 differs somewhat among the four samples of wheat flour included in this study. Hard red spring wheat gluten when dispersed in sodium salicylate has apparently the largest quantity of protein removed in fraction 1 following the addition of substantial quantities of enzyme to the doughs from which these glutes were initially washed. Hard winter wheat glutes have the least removed, although the soft winter results were quite close to the hard winter. The durum wheat gluten approached closest to the hard red spring in quantity of gluten in this fraction.

Fraction 2 is not greatly affected by the action of enzymes in the concentration employed in this work. Fraction 3 is increased in all the gluten dispersions by increasing the concentration of enzyme. This effect was not apparent, however, when yeast water was employed. This fraction increases in a striking manner when the results from the hard red spring wheat doughs are progressively compared to the hard red winter and the soft red winter values. The increase in fraction 3 for the durum flour dough glutes is second lowest, being next in order to the spring wheat. This change of protein quantity thrown down as fraction 3 appears to follow roughly the order of the bread wheat flours in protein content and malt-phosphate-bromate loaf volume. It may be that the effect of papain and pancreatin in concentrations employed in this study is to decrease the size of the gluten particle to a larger extent in the instance of the winter wheat than in the spring wheat. This would entail less protein being thrown down in fraction 1, which presumably contains the larger-size protein particles, than in the case of the winter wheat, with an increase in fraction 3 where a substantial portion of the small particles are removed. These shifts in the relative proportions or quantity of protein removed are not quantitative, however, and therefore can only be regarded as indicators or trends, and are not conclusive. It was previously found by one of the authors that durum wheat contained a lower proportion of fraction 1 and a larger proportion of fraction 3 than did hard red spring wheat of the 1936 crop. This is in accord with the present findings. A lower protein solubility was also postulated for the durum in that instance.

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## MAINTAINING A UNIFORM TEMPERATURE IN AN EXPERIMENTAL BAKING OVEN

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The maintenance of a uniform temperature and the reduction of heat loss in experimental baking ovens has been the subject of much attention by cereal chemists. The loss of heat is a factor of considerable importance, because the greater the loss the more difficult it is to maintain a uniform temperature in the oven and in the surrounding room. The rise in room temperature is particularly serious in areas of high summer temperatures and especially so if the baking laboratory is not air-conditioned. These considerations led to a study of heat losses in an experimental baking oven at this laboratory, and also to certain oven alterations that are believed to be desirable.

The inside dimensions of the oven are  $34 \times 34 \times 26$  inches high. The door opening is  $12\frac{1}{2} \times 18$  inches. A shelf rotating at 2 r.p.m. is suspended above a refractory hearth about one inch thick. Top and bottom heat is supplied by elements of 5 kw. total capacity. The removable front and a metal-lined door necessarily result in considerable metal connection between the inside and outside oven walls and in consequence the loss of heat is greatest from the front wall. The heat

<sup>1</sup> Hard Winter Wheat Quality Laboratory, in cooperation with the Kansas Agricultural Experiment Station, Manhattan, Kans.

losses consist of direct radiation and that occasioned by the frequent opening of the door. These two losses were found to be approximately equal. It was observed that opening the door for 10 seconds caused the temperature inside the oven to drop  $40^{\circ}$  F. and that  $1\frac{1}{2}$  minutes were required to restore the temperature.

In order to reduce the heat losses and to maintain a more uniform temperature the front and adjoining corners were insulated, the size of the door opening was reduced, and partitions on the rotor and a stationary canopy inside the door were installed. These modifications are illustrated in Figure 1. The insulation is about  $1\frac{1}{2}$  inches thick and

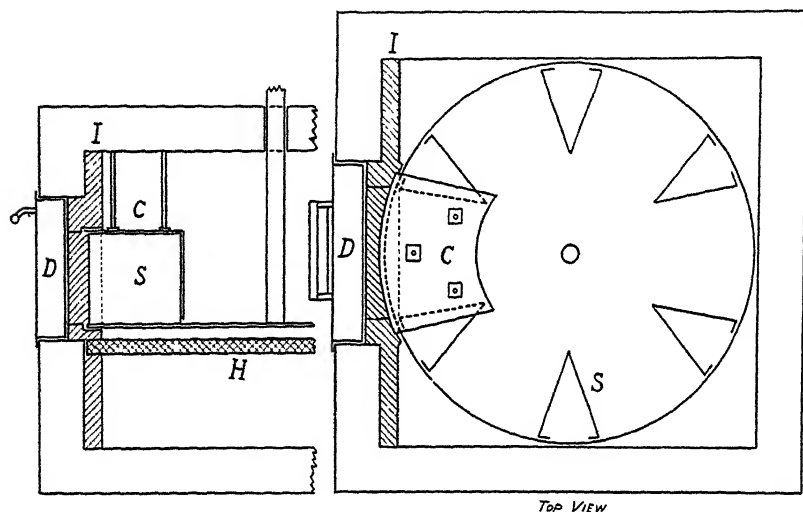


Fig. 1. Modified baking oven showing canopy (C), partitions (S), door (D), added insulation (I), and hearth (H).

consists of a shredded asbestos mixture containing some binder. It handles much like mud when wet. It has a low density when dry and sticks to the metal satisfactorily. The size of the door opening was reduced to  $8 \times 11\frac{1}{2}$  inches. The insulation produced a noticeable difference in the temperature of the outside walls of the oven and tests showed that the radiation losses were reduced by at least 25%.

The most important alterations were on the rotating shelf and these were adapted from equipment in several Canadian laboratories. In those ovens the rotating shelf contains a series of small, completely inclosed, identical sections, each large enough for a single loaf—essentially a series of ovens within an oven. The width of a section is the same as that of the door opening so that only one section is exposed when the door is opened. This arrangement reduces the heat loss con-

siderably but might be less desirable from the standpoint of experimental baking than the single large baking space.

After consideration of several possible plans to block off the major part of the oven when the door was open and yet allow all loaves to be baked in one large compartment, the device illustrated in Figure 1 was adopted. This consists of vertical side walls (*S*) fastened to the rotating shelf in such a way that they successively come under a stationary canopy (*C*) just inside the door. The canopy is suspended from the top of the oven and temporarily forms the top and back of each cell successively. When not under the canopy the loaves are separated from each other only by side walls and are baked under substantially uniform conditions. When the side walls of a section come into the position shown in the top view of Figure 1, the opening of the door exposes only a relatively small space to the exchange of air from the baking laboratory. The canopy and partitions were constructed of 14-gauge black iron. The canopy is attached to the top of the oven so that there is about  $\frac{3}{8}$  inch of clearance above the rotating shelf, which is reduced to about  $\frac{1}{8}$  inch or less when the oven is hot.

With this installation, opening the door for 10 seconds with the rotating shelf stopped, closing the door, and then starting the rotor caused an oven temperature drop of only 5° F., which was restored in a half minute. The heat lost to the room by opening the door in this manner once every three minutes did not cause any detectable increase in the power consumed by the oven. Stopping the rotating shelf at the proper position is easily effected by watching an indicator installed on the top of the oven which shows the shelf position. Tests have shown the variability in loaf volume due to position in the baking schedule to be lower than previously, and this can be at least partly attributed to the changes in oven design.

It was at first thought that the hearth supplied with the oven was of no appreciable advantage and should be removed, as many cereal chemists have done. However, it was found that the change in oven temperature from an empty to a full load was 6° F. with the hearth in place and 17° F. after the hearth was removed. Although the drop with the hearth in place was comparatively large for the first loaf, the additional drop from this point to a full load was only 2° F. as compared with 6° F. with the hearth out. Thus each test indicated that more uniform temperatures during the baking period could be maintained with the hearth in place.

### Summary

Insulation of an experimental baking oven effected a material reduction in heat lost by radiation.

The installation on the rotating shelf of a series of side walls which moved successively under a stationary canopy immediately inside the door reduced the heat loss still further and markedly improved the uniformity of temperature within the oven.

The temperature during the entire baking period from the first loaf to full oven load was maintained more uniformly with the refractory hearth installed than without it.

## A SIMPLE LABORATORY SHAKING MACHINE

MAX C. MARKLEY

Cargill, Inc., Minneapolis, Minnesota

(Received for publication September 19, 1938)

The new Zeleny method for the determination of the fat-acidity of corn<sup>1</sup> calls for the use of a shaking machine during the extraction of the fatty acids. Not many of the cereal laboratories are equipped with a shaking machine of sufficient capacity to handle any appreciable number of such tests at one time. No standard machine for shaking a large number of glass-stoppered bottles at one time could be found in an examination of the available laboratory supply catalogs. A Kahn-test instrument could be adapted to this purpose by constructing special bottle racks to replace the test-tube racks regularly supplied, but this would mean an investment of a hundred dollars or more.

A shaking machine of ample capacity can be constructed, without the utilization of special castings or costly machine work, from about \$20 worth of parts, including the motor, and not more than 16 hours of labor. Only hand tools are required though a power drill reduces the labor. This instrument can be built from angle iron and home-workshop power-transmission equipment. The bottle rack should be made of wood, preferably a rather soft and straight-grained wood such as redwood. The machine with a rack for holding 24 bottles is shown in Figure 1.

The base for the machine was built of  $1'' \times 1'' \times \frac{1}{8}''$  angle iron and measured 46'' long by 6'' wide. Either rivets or welds can be used in the framing. To this frame were bolted 8 line-shaft hangers with  $\frac{1}{2}''$  bronze bearings as shown in Figure 2. The sliding carriage was made of the  $1''$  angle iron and was  $17\frac{1}{2}'' \times 6''$ . It was carried on two 24'' lengths of  $\frac{1}{2}''$  steel shafting, spaced 5'' from center to center. The shafts were bolted to the carriage and slide freely in the four central bearings attached to the base.

<sup>1</sup> Lawrence Zeleny. Paper read at the 1938 convention.

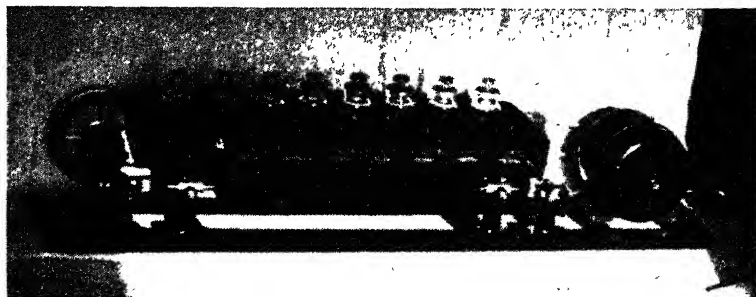


Fig. 1. Shaking machine fitted with rack for 24 bottles.

The crank was made from a solid steel pulley of  $\frac{3}{4}$ " face and  $1\frac{3}{4}$ " diameter. The crank-spindle was made by bushing a stove bolt which was passed through the pulley  $\frac{5}{8}$ " from the center. This gave a  $1\frac{1}{4}$ "

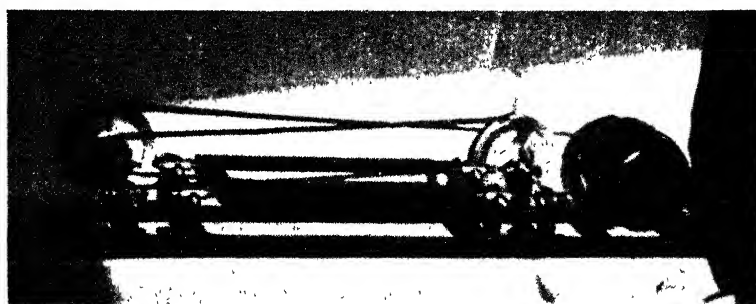


Fig. 2. Chassis of shaking machine showing crank assembly and carriage bearings.

throw to the pitman rod, which was made of  $\frac{3}{4}$ "  $\times$   $\frac{3}{4}$ "  $\times$   $\frac{1}{8}$ " angle iron with the ends filled with bearing metal. The pitman rod was quite long,  $19\frac{1}{2}$ " between centers, which insured smooth action. The crank-shaft was fitted with a  $6\frac{1}{2}$ " pulley which was driven from a  $2\frac{1}{2}$ " pulley on the counter-shaft. For a 1750 r.p.m. motor a  $1\frac{1}{2}$ " pulley was found to be satisfactory with a  $6\frac{1}{2}$ " pulley on the counter-shaft. With the long distances between centers the belt tension was low and round belts were satisfactory. The motor need not be over  $\frac{1}{6}$  horsepower.

# THE RELATION OF MECHANICAL STIRRING TO SPONGE DOUGHS

J. C. BAKER and M. D. MIZE

Wallace & Tiernan Laboratories, Newark, N. J.

(Read at the Annual Meeting, May 1938)

The authors have shown in a previous paper that the action of bromate in a dough is promoted by mechanical stirring. Upon observing a trough of dough during the sponge period it was noted that the evolution of gas and the extension of the dough by the bubbles kept the dough in a slight but continuous motion. In view of the effect of mechanical working of doughs upon bromate action, this motion in the sponge suggested that one of the functions of the sponge was the stimulation of the bromate by motion. Bearing in mind that the action of bromate can be accelerated mechanically, one might be able to substitute mechanical stirring for the fermenting sponge period in bread making and obtain similar results. This hypothesis was tested and is illustrated in the following table.

TABLE I  
BAKING RESULTS  
Kansas patent—ash 0.40%, protein 13.0%, moisture basis 15%

Mixing time <sup>1</sup>	Type of fermentation	Volume	Texture	Maturity
<i>min.</i>		<i>c.c.</i>		
3	2½ hrs. straight dough—no bromate	2260	80	Green
3	2½ hrs. straight dough plus 20 p.p.m. bromate	2540	95	Mature
3	4 hrs. 60%-40% sponge—no bromate	2480	86	Green
3	4 hrs. 60%-40% sponge plus 20 p.p.m. bromate	2730	97	Mature
3	4 hrs. 60%-40% sponge plus 20 p.p.m. bromate, no yeast in sponge—yeast added during dough-up	2520	80	Green
30	Mechanical 60%-40% sponge—no bromate, 20-minute fermentation	2340	88	Green
30	Mechanical 60%-40% sponge plus 20 p.p.m. bromate 20-minute fermentation	2760	97	Mature

<sup>1</sup> A Swanson mixer running at 52 r.p.m. was used.

The results without and with bromate in a straight dough are first given, followed by the results without and with bromate in a 4-hour sponge dough, showing the characteristic improvement in both of the sponge doughs, but the noticeably greater improvement produced by the bromate. The next loaf is a 4-hour sponge containing bromate in which the yeast was omitted, but subsequently added during the

dough-up. It is to be noted in this case that during the yeastless sponge stage where no motion occurs the resulting bread has practically the same volume as the 4-hour sponge without bromate and has the characteristic texture and quality of bread from unoxidized doughs, though the bromate had been present throughout the entire sponge and proofing period. In the absence of both yeast fermentation and mechanical motion, bromate has little apparent action.

The next two loaves indicated in Table I show the substitution of a 30-minute mechanical-mixing period for the 4-hour sponge period. In all other respects the handling of the doughs was the same and they were allowed the regular 20-minute resting period after doughing-up. It is to be noted where no bromate was used that the results were very similar to those obtained in the 4-hour sponge without bromate. Also where bromate was used, the results were very similar to those obtained in the 4-hour sponge with bromate. These results seem to confirm the theory that one of the chief functions of the 4-hour sponge is the activation of the bromate by the motion which the fermentation produces.

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## SOME OBSERVATIONS REGARDING THE FLAVOR OF BREAD

J. C. BAKER and M. D. MIZE

Wallace & Tiernan Laboratories, Newark, N. J.

(Read at the Annual Meeting, May 1938)

In connection with some of the researches being conducted in this laboratory, a method of baking crustless bread was developed. The dough was heated by being made the resistance between electrodes carrying alternating current. All portions of the dough were subjected to approximately the same temperature at the same time. No crust was formed. The bread was characterized by a slightly milder flavor, distinctly yeasty in character, suggestive of the odor of yeast fermentation—that is, slightly alcoholic but flavored with the other by-products of yeast fermentation such as diacetyl. The keeping quality of the flavor was superior to that of ordinary oven-baked bread. This crustless bread shows at the end of one, two, or three days of storage at room temperature very slight changes in the flavor characteristics, the main difference observed being a slight disappearance of flavor.

In contrast to this, bread freshly baked in the ordinary commercial way from the same dough exhibits a stronger flavor, rich in the odor of carmelization and the effects of high temperature on the crust. This odor is present not only in the crust but in the interior crumb of the bread. In other words, if the interior of the loaf is completely



removed from the crust and its odor observed separately it still carries the odor characterized by the crust, suggesting that the odors of the crust have been carried to the interior of the loaf.

Bread baked in the ordinary manner and also the separated interior portion of this bread develops upon storage the usual flavors which in the trade are called "stale."

A study was made to find what causes this flavor to develop in crust bread. A bread was made without shortening. A second bread was made in which ordinary shortening was used. A third bread was made in which a bland mineral oil was substituted for the shortening. A fourth bread in which ordinary shortening was used was thoroughly sprayed with mineral oil before panning and further sprayed before going to the oven.

The characteristics of these loaves, with an electric-baked crustless loaf containing shortening used as a standard, were as follows: The bread baked with mineral-oil shortening had a flavor nearly as mild as the crustless bread standard. The bread baked with ordinary shortening and sprayed with mineral oil had a flavor stronger than the standard but intermediate between mineral-oil shortening and ordinary shortening. The bread baked with no shortening had a flavor stronger than the standard but intermediate between the mineral-oil shortening and the ordinary shortening.

All observations on these loaves were made by six trained observers, all of whom are laboratory technicians familiar with cereal problems and familiar with bread flavors. All conclusions stated here are only those in which all observers were in agreement. In other words, the differences in the odor were so distinctly noticeable and so easily characterized that there was no disagreement among the observers as to these conclusions.

Upon storage the following observations were made: The bread with mineral-oil shortening retained during storage a flavor similar to the electric baked crustless loaf but with slightly more deterioration of flavor. The bread with no shortening and the bread with ordinary shortening but sprayed with mineral oil showed some deterioration of flavor and more evidence of flavor staling with age. The bread containing ordinary shortening and no mineral oil or spray gave the usual deterioration and developed the usual flavor staleness with age more definitely than any of the other loaves.

The following conclusions are evident from this work:

The flavor of bread is due to the flavor of the ingredients plus products developed by the yeast and to products developed by heat reactions in the crust.

The flavor products developed by the yeast and the ingredient flavor do not independently undergo appreciable deterioration with age and will not alone produce the stale flavor ordinarily found in old bread.

The flavor products developed in the crust during baking reach the interior of the loaf and are involved in the changes that occur in the flavor of the bread during aging and staling. These effects are intensified by the presence of shortening in the crust.

## ANNUAL REPORT OF TREASURER

OSCAR SKOVHOLT

January 1, 1939

The Association has continued to enjoy a healthy growth with a considerable net gain in both active and corporation members to a new high total of 600. The addition of 64 new members is a tribute to the activities of the Membership Committee.

The Executive Committee has authorized a transfer of \$200 per year (beginning July 1, 1938) from General Association funds for the support of *Cereal Chemistry*, to aid in securing more assistance for the management of the publication. This committee also decided that \$150 a year should be transferred annually to the Decennial Index fund, equally provided by *Cereal Chemistry* and the Association. The 1937 transfer eliminated the deficit in this fund and a surplus for the future indexes is now beginning to accrue.

An unprecedented surplus has resulted from the year's activities. Most of this surplus was earned by *Cereal Chemistry* due to increased receipts and reduced expenditures. The General Association profit is also substantial when considering that some expenses that are not annual were incurred. These include costs of the Osborne Medal Award and some expenses incidental to the transfer of the management of *Cereal Chemistry*. Reduced regular expenditures are responsible for the substantial surplus in the Association fund. All officers spent less than in previous recent years due to reduced expenditures for stenographic assistance. Since the time required to handle the details of all offices is increasing, this implies more work by officers and possibly more assistance by stenographers while on duty for the employers of the officials. It is believed that the time is at hand when more of the official duties should be delegated to paid employees of the Association.

The Secretary has placed an addressograph in working order and this expense plus certain addressograph services to other officers and committee members has been borne by that office. This undertaking requires considerable attention in view of membership and address changes, but one such address list may profitably be maintained by some Association official or employee.

On May 22d, the Executive Committee voted to transfer the remaining funds in the Experimental Laboratory Baking Fund to the Association General Fund. This transfer is shown in the statement under "Distribution of Net Assets."

The assets of the bankrupt Kansas City Building and Loan Company have been acquired by the North American Saving and Loan Association of Missouri, located in Kansas City. The Association assets in this Company now consist of \$400 of Class A stock with accumulated interest of \$18.26 plus \$1600 of Class B shares of undetermined value. The assets securing these Class B shares are being liquidated as rapidly as practicable, and when sufficient funds accumulate, they will be converted to Class A stock, which bears a  $3\frac{1}{2}$  per cent rate of interest at present.

There are on hand 140 copies of *Cereal Laboratory Methods*. During the year, 78 copies were sold, and the rate of sale is decreasing slowly. A revised edition may be desirable in 1941. A substantial surplus has accumulated to finance a new edition.

## DETAILED MEMBERSHIP STATEMENT DECEMBER 31, 1938

	Total	Active	Corp.	Hon.
Membership December 31, 1937 . . . . .	564	515	47	2
New members added during 1938 . . . . .	64	57	7	—
Members reinstated during 1938 . . . . .	6	6	—	—
Members resigned and suspended for non-payment of dues during 1938 . . . . .	30	29	1	—
Members lost by death . . . . .	4	4	—	—
Members in good standing December 31, 1938 . . . . .	600	545	53	2
Net increase in membership during 1938 . . . . .	36	30	6	—

## PROFIT AND LOSS STATEMENT

January 1 to December 31, 1938

## RECEIPTS

*Cereal Chemistry*

## Membership Dues

Active . . . . . \$1,914.50

Corporation . . . . . 530.00

Subscriptions, reprints, back issues, and advertising . . . . . 6,234.78

1938 Accounts Receivable . . . . . 333.25

Net 1938 . . . . . \$9,012.53

Interest on Invested Funds . . . . . 57.59

Miscellaneous Income . . . . . 3.50

Appropriated by Association . . . . . 100.00

Total Net Receipts 1938 . . . . . \$ 9,173.62

## Association

Membership Dues . . . . . 1,907.50

Application Fees . . . . . 171.00

Interest on Invested Funds . . . . . 53.85

Miscellaneous Income . . . . . 4.71

Total Net Receipts 1938 . . . . . 2,137.06

*Cereal Laboratory Methods* Sales during 1938 . . . . . 215.20

Interest on Invested Funds . . . . . 16.80

Total Net Receipts 1938 . . . . . 232.00

## Decennial Index

Received from *Cereal Chemistry* . . . . . 75.00

Received from Association . . . . . 75.00

Total Net Receipts 1938 . . . . . 150.00

TOTAL RECEIPTS OF ALL ACCOUNTS 1938 . . . . . \$11,692.68

## DISBURSEMENTS

*Cereal Chemistry*

Cost of printing Journal and Reprints . . . . . \$6,017.90

Less: 1937 account paid 1938 . . . . . 63.83

Net Cost of Printing . . . . . \$5,954.07

Cost of Editors and Miscellaneous Services . . . . . 1,809.32

1938 Accounts Payable . . . . . 6.34

Net Cost of Editing . . . . . 1,815.66

Decennial Index—*Cereal Chemistry* Assessment . . . . . 75.00

Net Disbursements 1938 . . . . . 7,844.73

Surplus 1938 . . . . . \$ 1,328.89

## Association

Expenses of President's and Vice President's Offices and News Letter . . . . .	257.12	
Expenses of Secretary's Office . . . . .	132.67	
Expenses of Treasurer's Office . . . . .	127.36	
Committee Expenses . . . . .	11.99	
Convention Ads in <i>Cereal Chemistry</i> . . . . .	20.00	
Cincinnati Convention Report . . . . .	331.06	
Osborne Medal Award . . . . .	317.55	
Expenses of Managing Editor's trip to Washington	91.25	
Appropriated to <i>Cereal Chemistry</i> . . . . .	100.00	
Decennial Index—Association's Assessment . . . .	75.00	
Miscellaneous Expense . . . . .	27.93	
Net Disbursements 1938 . . . . .	1,491.93	
Surplus 1938 . . . . .		645.13
<i>Cereal Laboratory Methods</i>		
Mailing Expenses . . . . .	18.13	
Surplus 1938 . . . . .		213.87
Decennial Index		
Surplus 1938 . . . . .		150.00
TOTAL DISBURSEMENTS OF ALL ACCOUNTS . . . . .		\$ 9,354.79

## DISTRIBUTION OF NET ASSETS

<i>Cereal Chemistry</i> Assets 1937 . . . . .	\$3,981.57	
Surplus 1938 . . . . .	1,328.89	
Assets Dec. 31, 1938 . . . . .		\$ 5,310.46
Association Assets 1937 . . . . .	3,818.54	
Transfer from Experimental Laboratory Baking Fund . . . .	80.95	
Surplus 1938 . . . . .	645.13	
Assets December 31, 1938 . . . . .		4,544.62
Convention Reserve Fund 1937 . . . . .	1,000.00	
Assets December 31, 1938 . . . . .		1,000.00
<i>Cereal Laboratory Methods</i> Fund 1937 . . . . .	1,070.29	
Surplus 1938 . . . . .	213.87	
Assets December 31, 1938 . . . . .		1,284.16
Decennial Index Fund 1937 . . . . .	None	
Surplus 1938 . . . . .	150.00	
Assets December 31, 1938 . . . . .		150.00
Experimental Laboratory Baking Fund 1937 . . . . .	80.95	
Deficit (by transfer) 1938 . . . . .	80.95	
Assets December 31, 1938 . . . . .		None
TOTAL ASSETS DECEMBER 31, 1938 . . . . .		\$12,289.24

## FINANCIAL STATEMENT DECEMBER 31, 1938

## ASSETS

Manufacturers Trust Company—Checking Account . . . . .	\$ 5,372.89
Petty Cash Fund—Lincoln, Neb. . . . .	199.18
Emigrant Industrial Savings Bank—New York . . . . .	851.24
Franklin Savings Bank—New York . . . . .	522.07
Harris Trust Company—Chicago . . . . .	2,016.95
Building & Loan Stock—Kansas City <sup>1</sup> . . . . .	1,000.00
U. S. Treasury Bonds . . . . .	2,000.00
1938 Income Receivable . . . . .	333.25
GROSS ASSETS . . . . .	\$12,295.58

<sup>1</sup> Carried on books at same value as authorized in 1936, although now consisting of \$400 of Class A interest-bearing shares in North American Savings and Loan Association of Missouri, with accumulated interest of \$18.26 plus \$1600 of Class B certificates of undetermined value.

GROSS ASSETS . . . . .	\$12,295.58
LIABILITIES	
1938 Accounts Payable . . . . .	6.34
NET ASSETS . . . . .	\$12,289.24

## REPORT OF THE AUDITING COMMITTEES

We have examined the books and the report of the Treasurer for the year 1938 and to the best of our knowledge and belief, these are a true and accurate account of the receipts and expenditures of the American Association of Cereal Chemists.

H. K. PARKER, *Chairman*

W. E. STOKES

CHAS. A. GLABAU

We have examined the books of the Managing Editor of CEREAL CHEMISTRY for the calendar year 1938 and find the same to be correct to the best of our knowledge.

H. H. JOHNSON, *Chairman*

A. A. ANDRE

## BOOK REVIEW

**Outlines of Biochemistry. The Organic Chemistry and the Physicochemical Reactions of Biologically Important Compounds and Systems.** By Ross Aiken Gortner, Professor of Agricultural Biochemistry in the University of Minnesota, and Chief of the Division of Agricultural Biochemistry, University of Minnesota and the Minnesota Agricultural Experiment Station. John Wiley and Sons, Inc. New York. Chapman and Hall, Limited, London. 1017 pages. Price \$6.00.

The appearance of the second edition of *Outlines of Biochemistry*, nine years after the first (for an excellent review of which see *Cereal Chemistry*, 6: 541), is enthusiastically welcomed by biochemical workers, teachers and students, whether in general or in highly specialized fields. All who have appreciated the nature, the quality, and the scope of the first edition will be impressed with the magnitude of the task of revision in the light of biochemical progress made during the past ten years, with particular reference to enzymes, hormones, vitamins, proteins, etc. As stated in the author's preface, "the present edition represents an extensive revision and in a large part the complete re-writing of the text. In addition, three new chapters have been added dealing, respectively, with oxidation-reduction, the flavins, and the hormones. A section on lignin has also been added." The chapter on vitamins is again contributed by Dr. L. S. Palmer.

As was true of the first edition, the book admirably serves the interests of both plant and animal biochemistry. The author's chief concern is with fundamental biochemical substances, properties, and processes *as such*, with special emphasis on colloid phenomena. The thorough discussion of colloids and colloid properties is justified on the basis that "all of the reactions and interactions which we call life take place in a colloid system."

Written in a style that characteristically reflects the author's forceful personality and enthusiasm, the book is unexcelled in its capacity for stimulating and holding the interest of the student. For illustrations and examples Dr. Gortner draws freely upon his large fund of personal experience and upon the experiences of his students and associates, both past and present. Numerous and well chosen references to the works of others are accompanied by full and complete literature citations. Although the current edition contains 200 pages more than the first one, the size of the book is substantially the same, and this without any sacrifice in quality of paper or printing.

The author has for many years maintained an active and a special interest in the basic constituents and colloidal properties of cereals, and his book contains numerous references to these and related matters. Gortner has been and remains a powerful influence in the lives and training of many cereal chemists, and it is safe to say that no wide-awake cereal technologist will want to be without access to a copy of *Outlines of Biochemistry*.

M. J. BLISH

# CEREAL CHEMISTRY

VOL. XVI

MAY, 1939

No. 3

## CEREALS AS A SOURCE OF VITAMIN B<sub>1</sub> IN HUMAN DIETS

R. R. WILLIAMS

Bell Telephone Laboratories, New York, N. Y.

(Received for publication January 21, 1939)

What are the constituents which *must* be present in their food if animals are to survive? This question has been posed and re-posed by physiology for a century. At times it has been presumed that a complete answer had been secured. Time and again, however, further and more detailed study revealed that earlier complacency was based on crudities of analytical methods, not upon genuine knowledge. Undoubtedly, the fact that animals are commonly observed to live successfully, at least for a time, upon widely differing food supplies contributed to the belief that they could have no very hard, fast, and detailed requirements.

In spite of a generation of intensive and skeptical examination of the question, it is still impossible to make a complete list of all the specific substances which animals require for the maintenance of health and vigor. The list already includes many mineral elements, such as calcium, magnesium, copper, iron, zinc, manganese, iodine, phosphorus, etc., in fact pretty much all minerals of sea water. Several specific amino acids, such as lysine and tryptophane, are recognized as essential, the amino acids appearing in the food as component parts of the proteins. All of these substances are found in the body as constituents of the tissues or body fluids. In addition, there is an already long list of vitamins, by which we merely mean specific organic compounds which are essential in small amounts to proper animal function and which must be supplied in the food, since in general animals have no capacity for synthesizing them. Some of these are fat-soluble substances, such as vitamins A and D; others are water-soluble and wholly different in character. The water-soluble ones include vitamin C (anti-scurvy vitamin) which contains only the elements carbon, hydrogen, and oxygen; vitamin B<sub>2</sub> (riboflavin) and nicotinic acid (anti-pellagra vitamin) contain also nitrogen, while vitamin B<sub>1</sub> (anti-beriberi vitamin) contains, in addition, a fifth element, sulfur.

The vitamins are accordingly very diverse in their chemical nature and exhibit great contrasts in their stability to heat, their distribution and abundance in foods, etc., so that few general statements can be made which are true of them all. That which is common to all of them, aside from their ultimate origin in plants, is a certain, at least apparent, similarity of physiological role. Even this similarity is somewhat vague and, as regards some groups of vitamins, conjectural. However, students of these substances have come in recent years to feel that many, if not all of them, will be found to be parts of enzyme systems, that is, catalytic mechanisms necessary for the conversion of external foodstuff into internal tissue constituents or into vital energy. In some cases, notably those of several of the B vitamins, evidence to this effect is already strong.

More and more it is becoming evident that the vitamins which occur in plant tissues are not present there as accidental components, but that they play roles in the plant world similar to those they play in the animal kingdom. The plant fabricates these substances for its own benefit. Their utilization by animals is a parasitic incident. The vitamins, therefore, constitute another example of the dependence of animals upon plants. As has long been recognized, the latter convert carbon dioxide to carbohydrates by the aid of sunlight and thus furnish an indispensable caloric supply for animals. In this phase the animals degrade the complex organic compounds, notably the carbohydrates, which the plants build up. The two kingdoms are thus in contrast. In another phase, that of the vitamins, the plants carry on many and varied organic syntheses of compounds which are similarly utilized by both plants and animals as mechanisms of cellular metabolism. With respect to these mechanisms, the two kingdoms exhibit kinship rather than contrast.

This evidence, that plants and animals utilize in common so many chemical mechanisms, adds a great emphasis to the idea of the essential unity of all forms of life. Both man and the potato on which he sometimes subsists have a common heritage from the earlier and more primitive forms of life from which they sprang. The same mass of evidence also adds to the marvel that life should have developed at all. If such a variety of intricate physiological mechanisms are necessary for the survival of even the simplest forms of life, what countless experiments Nature must have performed before the first viable cell appeared! What incredible combinations of circumstances must have surrounded each successive step of amplification of its initial powers!

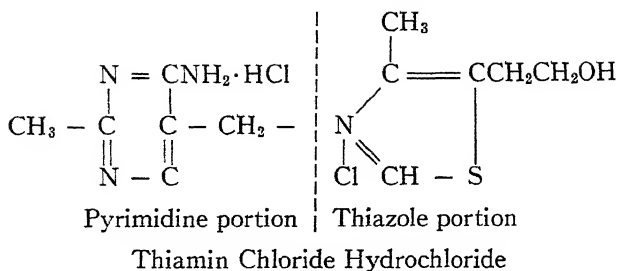
In the light of later events it seems likely that the nearly universal

occurrence and physiological role of the vitamins had much to do with delaying our discovery of them. Had these substances been peculiar to a few distinctive plant tissues, as is the case with quinine for example, deficiency diseases would have been so common as to have been recognized as such by the ancients. Almost any combination of ordinarily available foods contains some of all of the vitamins (or their precursors) because the foods, being living tissues, required their presence to exist. So it was only under rather exceptional circumstances that deficiency diseases appeared in human beings. Thus sailors on long cruises, soldiers in beleaguered camps, and arctic explorers at a distance from their base of supplies fell a prey to scurvy because their rations were unusually restricted. Likewise, beriberi became prevalent in the Orient only as increasing population density restricted the people more fully to a rice diet and the advent of factory milling of the rice deprived it more fully of its external coats. In short, primitive man was protected from outright deficiency disease largely because he had a common descent and, in part, a common physiology with the plants which he or his animal prey used as food. That such is the case will become clearer from the account given below of the physiological role of vitamin B<sub>1</sub>.

~ It must be regarded as highly significant that early studies of vitamin B<sub>1</sub> were associated almost wholly with the human disease beriberi. Takaki largely eradicated the disease from the Japanese navy more than fifty years ago by substituting beef and barley for a part of the polished rice of the ration. Thus decorticated grain was condemned by practical experience long before laboratory men began to inquire as to the specific reason. Not until ten years later did Eijkman in Java reproduce the disease experimentally in chickens by feeding them on polished rice. He thus initiated the long series of studies in many lands, which progressively showed that the bran coats of grains contain in very small amounts a substance which is a specific preventive and cure for the disease. Thirty years after Eijkman's work began the pure substance was at last isolated by Jansen and Donath in the same laboratory in Java where their fellow countryman had labored. The amounts obtained were so small that even the elementary composition of the substance was not correctly determined at that time. Seven years later larger yields were obtained by Williams and his associates in America, and the work of determining its structure began.

It turned out to be a unique compound, consisting of a pyrimidine and thiazole having the following formula:





Its synthesis followed almost immediately. The whole matter has since been confirmed by British, German, and Japanese workers and commercial production of the substance has already reached hundreds of kilos. The substance has recently been given the name thiamin to identify it as the sulfur-containing vitamin.

No sooner had the synthetic substance become available for experimental work than evidence began to pour in from many sources regarding the nature and extent of its role in living things. First we may mention the discovery of Lohmann and Schuster that thiamin pyrophosphate occurs as such in yeast and is identical with the previously postulated cocarboxylase. This compound is a coenzyme, that is, a component part of an enzyme of yeast which is essential to the production of carbon dioxide from sugar. More specifically the enzyme functions at a particular stage of the fermentation process, involving the conversion of pyruvic acid,  $\text{CH}_3\text{COCOOH}$ , to acetaldehyde and carbon dioxide.

Thiamin, either free or in the form of its pyrophosphate, plays a similar, though perhaps not quite identical role, in the metabolism of animal tissues. Peters has long since shown that the brain tissue of beriberi pigeons cannot respire properly in a pyruvic acid medium. On addition of thiamin to the medium the brain tissue promptly consumed oxygen and gave off carbon dioxide more rapidly than prior to the addition. With thiamin present it behaved much more nearly like normal pigeon brain tissue and was able to utilize pyruvic acid, which otherwise accumulated in the tissues as a result of the unfinished metabolism of sugar. The impairment of pyruvic acid utilization in beriberi is not peculiar to brain tissue. Kidney, heart, and liver may show it, and it is fair to assume that it occurs in the cells of all organs in greater or less degree. It is of interest to note that pyruvic acid has since been found in abnormally large amounts in the blood and urine of people suffering from beriberi.

It seems a far cry from yeast cells to human tissues. Actually, they are not as far apart as it would seem, for a great mass of evidence

has accumulated indicating that the metabolism of sugar follows a more or less common pathway in all living things. Thus, many of the long series of steps by which glucose is converted to carbon dioxide and water in the yeast cell, are present as part of the process of contracting muscle or functioning nervous system in animals. There is reason to believe that in all living things the first step in the metabolism of glucose is its conversion into phosphoric ester, and this is followed by a splitting of the six carbon atom chain of the sugar into two phosphorylated fragments, each of three carbon atoms. These fragments lose phosphoric acid and are converted into pyruvic acid, a substance which is therefore to be regarded as a well-nigh universal intermediate in the process of sugar utilization. The course of the process beyond that point is more debatable, but it seems very likely that the availability of thiamin will be of great assistance in solving the problem, because it is at this stage of carbohydrate metabolism that thiamin evidently enters as a component of one or more necessary enzyme systems. Apparently the process can go normally thus far without thiamin, but halts badly at this point.

Since most living things need to metabolize carbohydrate at one stage or another of their life cycles, it is not surprising that a great many organisms have been found to require thiamin for their normal function. Thus, lactic acid bacteria contain it and use it as a part of their life process. This is equally true of staphylococcus, a common pus-forming organism. Many saprophytic plants, that is, plants which live upon the dead remains of other organisms, rather than solely upon inorganic substances, depend upon the thiamin which is present in these organic remains. Therefore, the thiamin requirements of many molds and fungi have been investigated with definite results. Certain fungi have even been proposed as a possible means of determining the amount of thiamin in a food extract or body fluid. Very generally it has been proved to be the case that organisms grow in proportion to the amount of thiamin which is present. Even yeast, which is well known as a rich source of thiamin, will grow much more thriftily in the presence of an ample supply, and will exhibit the power to ferment sugar correspondingly more rapidly. It does appear that yeast has a certain capacity to synthesize thiamin, although I doubt whether the evidence is quite beyond dispute. Be that as it may, it is certainly true that the yeasts which are richest in thiamin are those grown in media which contain a great deal of it. The fact that beer is almost free from thiamin, even though the original wort was rich in it, is evidence of the great avidity with which the yeast cell seizes any available supply of the substance and incorporates it within itself.

As the studies of the effect of thiamin on the growth of a wide

variety of plant tissues has proceeded, the interesting observation has been made that the intermediates in the artificial synthesis of thiamin will sometimes serve as a substitute for the finished product of synthesis. Thus, some plants or plant tissues require the thiazole portion of the vitamin in order to grow, others more conspicuously need the pyrimidine portion, still others require a mixture of the pyrimidine and thiazole portions. Only a few plants have been found which require the complete molecule in order to carry out their normal processes. This observation has been of great aid in making a survey of the synthetic capacity of living things. The survey is, of course, as yet a very preliminary one, but it indicates at once a well-nigh universal need for thiamin in plant tissues and a highly variable capacity to synthesize it. We must suppose that those which require only the thiazole portion are able to synthesize the pyrimidine portion, and *vice versa*. Some can synthesize neither of the two portions, but show an ample capacity to put the two portions together to produce the final product, thiamin. Thus, many plant tissues fail at one stage or another in ability to synthesize a thing which they all need. We know this from experiments which involve the growing of one organism successively after another in the same identical lot of medium. Thus, *Phytophthora* grows readily in a medium in which *Phycomyces* has grown, although the former is entirely unable to grow in the medium as freshly prepared.

In the light of what has been said above, a well-nigh universal occurrence of thiamin in all foodstuffs becomes highly significant. A little of it is present in all live things because it is necessary to their life processes. Even polished rice and white flour contain measurable amounts of it. It is also highly significant that very few foodstuffs contain much of it. This we believe is a reflection of the fact that the capacity for the synthesis of this substance is rather limited. Living things, in general, seem able to make very little more of it than they need for their own growth. To what extent this is due to the instability of the substance once it is formed, and to what extent it is due to meagerness of synthesis is unknown, but it does seem very clear that the accumulated supply of Nature is marginal. This is certainly generally true in the animal world, as there is no very extensive storage of the substance in the body. Given a rich supply of the food the excretion in the urine rises correspondingly, and only a few weeks of feeding on a thiamin-poor diet is sufficient to deplete the body of its reserve and bring on beriberi. Accordingly, the appearance of this deficiency disease so early and so universally among animal species is an indication of their spendthrift character in this regard. The speed of onset is roughly proportional to the metabolic rate and is

thus greatest in the smallest animals. These metabolize food rapidly in order to offset loss of body heat from the skin surface.

If, as we have seen, various plants and micro-organisms differ with respect to their capacities for synthesizing the components of thiamin and for effecting the final union of these components, it might also be expected that a given plant would exhibit variations in synthetic capacity at different stages of its life cycle. Indeed, it is only under highly artificial conditions, such as, in purified media, or by the use of excised portions of plants, that their needs for thiamin can be demonstrated. The whole plant growing under natural conditions usually finds thiamin in its surroundings. But if we isolate a properly chosen bit of plant from external sources of thiamin, we find the need existing. This is conspicuously true of the higher plants. The evidence is that the higher plants synthesize the vitamin in their green leaves but store only limited quantities of it there. The leaves constitute the source of supply for the roots and other parts, and their synthetic capacity corresponds roughly to the growth impulse in them. Thus, the shoots of fresh young grasses contain more thiamin than those of old mature plants, which are approaching the end of their season.

This brings us to the particular theme which will be of special interest to the cereal chemist. It is well known, of course, that higher plants store a reserve supply of food in their seeds to enable the young seedling to grow until it has established itself in its new environment, by putting forth roots into the soil and pushing shoots up into the air and sunlight. Some seeds store fats, but in many instances the reserve food supply takes the form of starch. This is conspicuously true of cereals, the seeds of which, by a long process of artificial selection, have been developed to contain a maximum of this component. Their external parts are richer in thiamin than any other plant (or animal) tissue.

Starch, being a carbohydrate, inevitably passes through the glucose stage in its metabolism. For the metabolism of this glucose, we should confidently expect that thiamin would be required. It is, therefore, almost unmistakable that Nature's reason for a high content of thiamin in seeds is that they also have a high content of starch which the germinating plants must metabolize. This has been amply demonstrated for a limited number of plants by their cultivation in artificial media. Excised pea embryos, although they contain a little thiamin, will grow very slightly in a sugar solution. Too large a part of the thiamin has been discarded with the rest of the seed. Add thiamin to the medium and the growth is multiplied several fold. Tomato roots furnish even more striking experimental material. For this purpose allow tomato seeds to germinate in a pure sugar solution. When the

rootlets have formed, cut off their tips, and transfer the tips to a new flask of sugar solution. They stop growing, not because they have been severed from the seed but because they can no longer draw upon it for thiamin. Add synthetic thiamin to the new flask and copious growth of the roots will occur apart from the seed, in proportion, over a wide range, to the amount of thiamin added.

There is here, we confidently believe, a profound bit of philosophy regarding the selection of human food. When one eats eggs or oysters, one takes advantage of everything that the living organism contains. The very fact that the egg or the oyster has grown and developed and is capable of further and independent growth and development is evidence that it contains all that, at least a restricted, life requires. Eat liver, kidney, or pancreas and one gets the products of varied glandular activity. Eat milk and one gets the entire natural food supply of the young. Eat leaves and one is consuming tissue that yesterday, or a week ago, was in the active process of growth and, accordingly, of thiamin synthesis. Eat the whole seed and one assimilates the entire organism which, while it is for the moment at a dormant stage, is still demonstrably capable of a lively development. But, carefully to sift the starch from the other parts of the seed and make the starchy endosperm the chief source of energy for human life, is to fly in the face of Providence.

By fortunate accident there are few foodstuffs which lend themselves so readily to sterilizing purification. One may, as the Eskimos do, eat whale blubber, or one may try out lard or express corn oil for human consumption, but that is not so bad, for the purified foodstuff, being fat, does not require thiamin for its metabolism. Only the producer of refined cane sugar can rival the miller of rice or wheat (the world's two largest cereal crops) in the thoroughness of the devitalizing purification process.

Unquestionably, from a long range business standpoint, the greatest opportunity for the miller, and the sugar refiner as well, is to find a way to meet popular demands without continuing to call upon their customers to disregard Nature's laws, or to make up the deficiencies of the carbohydrate staples by judicious use of other foods. One cannot of course ignore the popular fancy for white bread, nor the concrete fact that whole cereals or their mill products are more subject to spoilage than the whiter forms (in part, for the excellent reason that the latter will scarcely support bacterial or insect life). One cannot wholly ignore the testimony of qualified physicians, that branny roughage is irritating to the intestinal tracts of some people, though that is probably more a matter for the hospital dietician than for the housewife. All these are parts of the large problem which the carbohydrate

industries face, that of making their staple products more nearly the equivalent in nutritive value of the whole seed or the cane stalk, as it was once consumed by primitive man. Whether this is to be done by additions of synthetic materials or by retention of the original nutritive components of the crude foodstuffs is a question for industry to decide. To blink at the scientific facts, which will presently become common knowledge, will be suicidal for the commercial enterprises concerned.

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## STUDIES ON WHEAT STARCH. I. THE AMYLOPECTIN AND AMYLOSE CONTENT OF VARIOUS WHEAT STARCHES<sup>1</sup>

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### Constituents of Starch

Careful analysis of starch reveals that it is not a homogeneous substance, but that noncarbohydrate constituents such as phosphorus, fatty acids, nitrogenous material, and silicon are also present in small amounts in the different kinds of starches.

The presence of phosphorus in starch is generally conceded, but its distribution in the starch granule has been the object of much controversy. Samec (1934) suggested that phosphoric acid in starch is in some manner combined with the nitrogenous substance and united with the polysaccharide in the form of "phytovitellinen." Koets (1935) suggested that the amylopectin fraction of starch is a complex coacervate of amylophosphoric acid and a nitrogenous substance, probably of protein character. Samec and Beniger (1931) reported that at least a portion of the nitrogen in starch occurs in chemical combination. Samec (1934) believes that in some starches the phosphoric acid is present as a complex silicophosphate which in wheat starch is deposited as an insoluble salt, for example, a calcium salt, and in potato starch as a soluble potassium salt. Taylor and Lehrman (1926) found that the fatty acids of corn starch consist of approximately 24% palmitic, 40% oleic, and 36% linoleic, and (1930) that those of wheat starch are about 35% palmitic, 41% oleic, and 24% linoleic.

The starch granule consists chiefly of two carbohydrate constituents. Meyer (1895) introduced the names alpha-amylose for one portion and beta-amylose for another portion of the starch granule.

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Maquenne (Walton, 1928) used the term amylopectin instead of alpha-amylose, and amylose instead of beta-amylose. Both terminologies are now used in the current literature. Samec and coworkers (Samec and Mayer, 1921; Samec and Waldschmidt-Leitz, 1931) prefer the names amyloamylose and erythroamylose for two starch fractions obtained by electrophoresis, with the erythroamylose migrating to the anode.

Samec and Haerrdtl (1920), Samec and Mayer (1921), Samec (1934), Sherman and Baker (1916), Taylor and Walton (1929), Baldwin (1930), Kavcic (1930), and Stamberg (1936) found practically all  $t^1$  phosphorus in the amylopectin fraction. Other investigators including Karrer and Kraus (1929), Taylor and Iddles (1926), Hirst Plant, and Wilkinson (1932), and Taylor and Schoch (1933) found that both starch fractions contained about the same amount of phosphorus. Widely different methods of fractionation were used by these investigators and incomplete separation in some instances would lead to such differences in results. Taylor and co-workers found the fatty acids of starch entirely in the amylopectin fraction and considered the presence of fatty acids rather than phosphorus to be the primary difference between amylose and amylopectin.

It is generally agreed upon by various investigators that the amylose is quite soluble in water, has a lower viscosity than amylopectin, and gives a blue iodine color, and that the amylopectin is less soluble, has a higher viscosity, and gives a red-violet color with small amounts of iodine.

### Structure of Starch

The presence of a membrane around the starch granule is frequently referred to in the starch literature. If such a membrane exists, it is probably not the amylopectin fraction. The present evidence points to a fairly uniform distribution of amylose and amylopectin throughout the granule, and that the outer portion is in a less hydrated state. Morris (1934) suggested that a membrane of less hydrated or retrograded amylose might exist while the starch granule is yet in the plant. Sande-Bakhuyzen (1925) observed that the surface was the most dehydrated part of the starch granule. Alsberg (1937) stated that "The observations of Lynst-Zwicker, 1921, which the writer has been able to confirm, indicate that the membrane of gelatinized granules is an artifact formed during gelatinization by accumulation of less soluble materials at the periphery of the granule."

Hanson and Katz (1934) found that potato and wheat starches, following a treatment with hydrochloric acid and calcium nitrate, were divided radially and tangentially into small blocks of about  $1\mu$ , which

were separated by some other substance. They assumed that the blocks were the amylose and that the amylopectin was the substance interposed between them. The same block structure was observed by Badenhuisen (1937, *a* and *b*) in wheat starches, but he concluded that the blocks appeared only upon gelatinization of the granules. Nikolaieff and Schultz (1933) suggested that the wheat starch granule is made up of two lens-shaped halves held together by radially oriented micelles. Sande-Bakhuyzen (1926) grew wheat under constant artificial illumination. The starch granule did not have the rings characteristic of ordinary wheat starch, and radial needles resembling pyramids with a base of  $2-3\mu$  were observed in the granules.

As to the chemical structure of the starch molecules, Hirst, Plant, and Wilkinson (1932) give the well known Haworth model of 24 to 30 glucopyranose units for both amylopectin and amylose, stating that in amylopectin the molecules are denser and more interlocking than in amylose. Staudinger and Husemann (1937) studied the properties of starch and starch derivatives by viscosity, cryoscopic, and diffusion methods. They stated that the Haworth straight chain model of starch does not explain the difference in diffusion constants obtained by starch and cellulose. They suggested that the starch molecule includes a main chain with some side chains linked through their aldehyde groups to the main chain, thus producing fewer free reducing groups. Caldwell and Hixon (1938) concluded after a series of periodic acid oxidation studies of several starches and dextrans that the average chain length of the starch molecule must be considerably greater than the 25 glucopyranose units estimated by Haworth.

Myrbäck and Ahlborg (1937) were in agreement with Staudinger and Husemann that the starch molecule is probably a branched chain system, and Myrbäck (1937) suggested that the reason for the incomplete hydrolysis of starch by beta-amylase might be found in certain anomalies in the starch molecule such as branch chains, or phosphorus and fatty acids in ester linkages. He suggested that further studies of the limit dextrans resulting from enzyme hydrolysis of starch will probably show linkages of glucose units other than the typical maltose linkage. In this connection it is interesting to note that in the diagram by Staudinger and Husemann with the branched chain linked through the number 6 carbon of the main chain glucose unit, there is a possibility of obtaining gentiobiose (glucose, 6,  $\beta$ -glucoside) from starch. Berlin (1926, *a* and *b*) actually reported the isolation of gentiobiose from the residue commercially known as "hydrol" obtained in the manufacturing of *d*-glucose from corn starch. He also demonstrated that gentiobiose is identical with Fisher's (1896) isomaltose produced by the action of strong hydrochloric acid on glucose. Since acid



hydrolysis is used in commercial preparation of glucose from starch and since gentiobiose is identical with Fisher's isomaltose, the question remains whether the gentiobiose obtained by Berlin was a residue from the starch or a product of synthesis. Further studies on the dextrans from starch with its probable "anomalies" will undoubtedly furnish valuable clues as to the structure of the starch molecule.

### Experimental

*Preparation of the starches.*—Five different wheats were used for the preparation of starch and Table I gives their source, crop year, and bushel weight. A Thatcher wheat of normal and one of low bushel weight were included. The wheat samples were milled in the experimental mill and flours of about 80% extraction were prepared.

In an effort to retard the diastatic action, small doughs were mixed at 5° C., using the flour and a 2% sodium chloride solution at the same temperature. After 20 minutes the starch was washed free from most of the gluten over a fine sieve. The starch was recovered by means of

TABLE I

Wheat	Source	Crop year	Bushel weight
Little Club ( <i>T. compactum</i> )	Pullman, Wash.	1936	62 lbs.
Federation ( <i>T. vulgare</i> )	Pullman, Wash.	1936	62 lbs.
Durum ( <i>T. durum</i> )	Minnesota	1936	59 lbs.
Thatcher ( <i>T. vulgare</i> )	Minnesota	1936	56 lbs.
Thatcher ( <i>T. vulgare</i> )	Minnesota	1935	48 lbs.

a cup centrifuge, resuspended in distilled water at 5°–8° C., and precipitated again in the centrifuge. This was repeated twice at the same temperature, and three to five times at room temperature. The protein content of the flours, the starch yield, and the nitrogen content of the starches are shown in Table II. A sample of commercial wheat starch was also included.

*Separation of small and large granules.*—Alsberg (1937) separated the small and the large starch granules by using an air classifier. The principle of this method is that in a constant flow of air the smaller granules will rise to a higher level than the larger. This method was tried, but the difficulties encountered in dispersing the granules and the larger quantities of starch required made it unsuitable.

For this study the small and the large granules of starch Thatcher 56 and commercial starch were separated by means of a specially built sedimentation column. The sedimentation column employed was 15 cm. in diameter and 120 cm. high with three dampers which could be turned, thus dividing the column into four equal parts. The lower

TABLE II  
PROTEIN CONTENT OF THE FLOURS—YIELD AND NITROGEN CONTENT OF THE STARCHES

Material	Protein content of flour (N $\times$ 5.7), 15% moisture basis	Yield of starch, air-dry basis	Nitrogen content of starch, moisture-free basis
	%	%	%
Little Club.....	6.37	59.5	0.048
Federation.....	8.70	56.8	0.035
Durum.....	15.81	44.0	0.045
Thatcher, 56 lbs. per bushel.....	13.99	52.7	0.056
Thatcher, 48 lbs. per bushel. ....	16.17	38.7	0.036
Commercial.....	—	—	0.054
Thatcher, 56 lbs., small granules... .	—	—	0.056
Thatcher, 56 lbs., large granules....	—	—	0.042
Commercial, small granules.....	—	—	0.051
Commercial, large granules.....	—	—	0.052

parts were filled with distilled water and into the upper part was added a suspension of 100 g. of starch. The dampers were opened and the sedimentation was allowed to proceed for almost an hour when the dampers were closed and each starch fraction was removed through spigots and then recovered by means of the cup centrifuge. Some 15 to 20 refractionations were necessary for each 100 g. of starch before a fairly pure fraction of small and one of large granules were obtained. No attempt was made to make quantitative separations. Some toluol was used in the water to prevent bacterial action. The nitrogen contents of the two final starch fractions are included in Table II. The photomicrographs in Figure 1 show the original Thatcher 56 starch and the small and the large granule fractions. The majority of the large granules were from 20 to 35 $\mu$  in diameter and the small granules from 3 to 6 $\mu$  in diameter. The fractions from commercial starch were similar to those shown in the photomicrographs.

*Grinding of the starch.*—The starch granules must be thoroughly disintegrated before they can be successfully separated into amylopectin and amylose by the electrophoretic method. Taylor and Keresztesy (1936) showed that the amount of amylopectin obtained from corn starch depended upon the length of grinding previous to the separation by the electrophoretic method. Using a ball mill, they found that 168, 336, 672, and 1,344 hours of grinding gave 18.6, 14.5, 8.1 and 6.2% of amylopectin, respectively. This indicates that the amounts of amylopectin and amylose thus separated are relative rather than absolute, and the percentages depend upon the degree of disintegration of the granules.

A rod mill was found to have a much more rapid grinding action than a ball mill. A one-gallon ball-mill jar with thirty steel rods

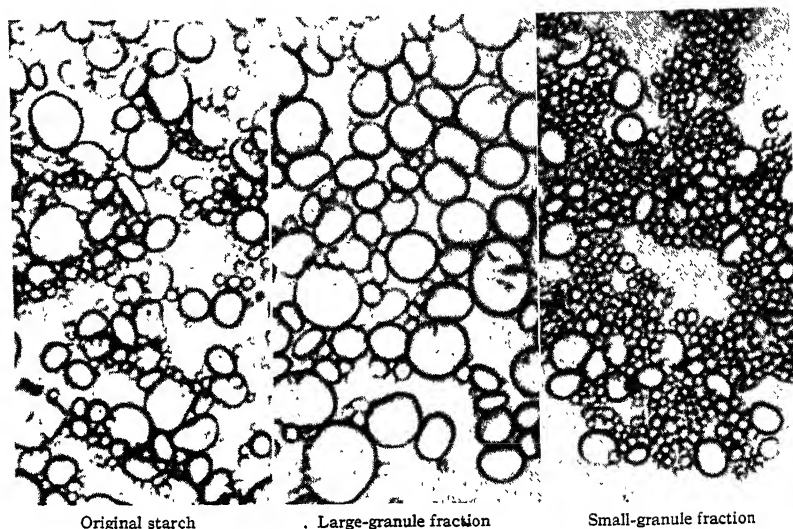


Fig. 1. Photomicrographs of Thatcher 56 wheat starch.

13 cm. long and 2 cm. in diameter was rotated at the rate of 14.8 r.p.m. All samples were ground for 84 hours, which according to microscopic observations was sufficient to thoroughly disintegrate all the starch granules. Samples of 100 g. each were ground except for the small- and large-granule fractions, of which only 75 g. samples were available.

*Separation of the amyloses.*—The electro dialyzing apparatus used in the separation of the amyloses was a slightly modified type of that described by Taylor and Iddles (1926). It is shown by the diagram in Figure 2. The bottoms of jars A and B were collodion membranes deposited on cheese cloth. Leaking membranes must be guarded against. Hard carbon electrodes were used, and a direct current potential of 220 volts.

Exactly 1,000 c.c. of 1.1–1.3% boiled solution of the pulverized starch was introduced quantitatively into jar B. The electrodes were placed in position and the current was turned on. After three days the migration was complete and the amylopectin formed as a slimy layer at the anode membrane on the bottom of jar B. The supernatant liquid was almost clear. It was carefully siphoned off into a liter volumetric flask and made up to volume by distilled water. A 50 c.c. aliquot was dried at 110° and used to determine the amount of amylose removed. The amylopectin layer was redispersed in a liter of hot water, and upon cooling was subjected to another electrophoretic separation. This was repeated about six additional times to remove all of the amylose as determined by the absence of weighable solids

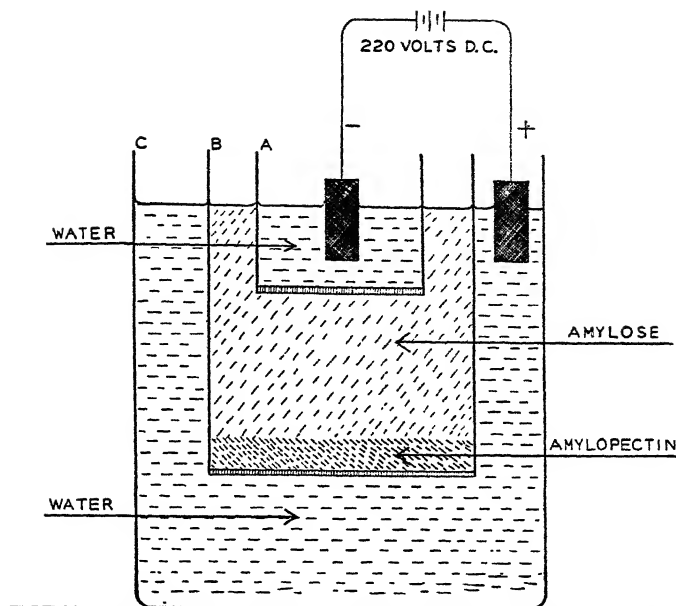


Fig. 2. Diagram of the electrolysizing apparatus. Necessary clamps and holders are not shown. Dimensions of the cells: A, diam. 10 cm., ht. 10 cm.; B, diam. 14 cm., ht. 19 cm.; C, diam. 26 cm., ht. 30 cm. Collodion membranes on cheese cloth.

in the aliquot from the supernatant liquid. The percentage of amylopectin was determined by the difference between the amount of starch used and the amylose removed. A few drops of toluol were always added to the starch solutions to prevent bacterial action. The percentages of amylopectin from two separations of each starch sample and the average values are given in Table III.

TABLE III  
RESULTS ON AMYLOPECTIN AND PHOSPHORUS DETERMINATIONS

Starch used	Grinding of starch in rod mill		Amylopectin			Phosphorus in			Total phosphorus of starch in the amylopectin
	Hours	Amount	1	2	Av.	Starch	Amylose	Amylopectin	
			%	%	%	%	%	%	
Little Club...	84	100	15.15	14.99	15.07	0.050	0.002	0.238	71.8
Federation...	84	100	16.92	15.02	15.97	0.059	0.002	0.387	102.5
Durum.....	84	100	16.26	16.10	16.18	0.070	0.003	0.404	93.3
Commercial...	84	100	17.13	17.38	17.26	0.061	0.001	0.261	73.8
Thatcher, 56 lbs. per bu.	84	100	15.36	14.31	14.84	0.068	0.001	0.354	77.2
Thatcher, 48 lbs. per bu.	84	100	15.21	16.00	15.60	0.073	0.004	0.437	93.2
Commercial, small granules...	84	75	15.79	14.85	15.32	0.057	0.001	0.293	78.8
Commercial, large granules...	84	75	14.45	15.08	14.76	0.055	0.004	0.344	92.3
Thatcher, 56 lbs., small granules...	84	75	13.32	12.58	12.95	0.068	0.007	0.483	92.0
Thatcher, 56 lbs., large granules...	84	75	11.82	11.97	11.90	0.061	0.007	0.436	85.1

*Phosphorus determinations.*—Since the phosphorus content of amylopectin and amylose and large- and small-size starch granules have been problems of interest, phosphorus determinations were made by the method described by Morris, Nelson, and Palmer (1931). The results are shown in Table III, and also the calculated percentages of the total phosphorus of the starch recovered in the amylopectin fraction.

### Discussion of Data

In view of the observations of Taylor and Keresztesy (1936) that the amount of amylopectin obtained is only relative and depends on the degree of disintegration of the granules, the values obtained must be considered as relative and not absolute until the two starch fractions have been more thoroughly defined. The percentages of amylopectin obtained from the six kinds of wheat starches appear to be about the same, and the small differences are probably not significant and are well within the limits of accuracy of the method.

The percentages of amylopectin recovered from the small and large granule fractions indicate that there is probably no difference due to granule size. The separation of the large and the small granules was sufficiently complete (Fig. 1) so that any differences due to granule size would be expected to be quite large. The amylopectin contents of the Thatcher 56 small and large granules were lower than those of other samples, but the higher phosphorus content of the amylose fractions indicates that the fractionation was probably incomplete and that some amylopectin was collected with the amylose fraction. These results are in agreement with the theory that amylopectin is distributed quite uniformly throughout the granule; otherwise, if the outer membrane were amylopectin, the percentage for the small granule would be much larger because of the significantly greater surface area of the small granules.

Grüss (1932) separated the large and the small granules from barley starch by the sedimentation method. Starch pastes were then subjected to a continuous filtration at 60° C. through a porous porcelain filter. The starch in the filtrate was precipitated by an excess of tannin and the precipitate was dried and used as the value for the amylose. By this method, he found 12.8% amylopectin in the large granules and 34.8% in the small granules. The membranes of the gelatinized starch granules probably remained in the filter—hence the larger value for the small granules with the greater amount of membrane.

The phosphorus contents of the six kinds of starches were much in the range found by many previous workers. Practically all the

phosphorus was found in the amylopectin fractions, with only traces remaining in the amylose fractions.

The phosphorus content of the small and the large granules appeared to be approximately the same, and the small differences are not significant in view of the relative completeness of separation of the large and the small granules. This agrees with Alsberg (1937), who separated different sizes of granules from tapioca starch and found that the fractions had much the same phosphorus content. It is further in agreement with Taylor and Schoch (1933) who found that the phosphorus is distributed quite uniformly throughout the granule rather than at the surface. Fernbach (1904) found the small granules from potato starch to be higher in phosphorus than the large granules from the same sample however, and Kavcic (1930) also found that in potato starches there was a tendency for the phosphorus to increase as the granule size decreased.

### Summary

Wheat starches from Little Club, Federation, Thatcher of 48 lbs. bushel weight, Thatcher of 56 lbs. bushel weight, and a durum wheat were prepared. These starches and also a commercial wheat-starch sample were finely pulverized in a rod mill, and subsequent fractionations by the electrophoretic method indicated that these starches contained practically the same relative amounts of amylopectin of from 15 to 17%.

Small and large wheat-starch granules separated from the same sample by a sedimentation process were found to contain about the same relative amounts of amylopectin as determined by the electrophoretic method.

There was no significant difference in phosphorus content of the small and the large granules separated from the same samples of wheat starch.

The amylopectin fraction separated by the electrophoretic method contained almost all of the phosphorus of the starch, and only a trace of phosphorus was found in the amylose.

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## STUDIES ON WHEAT STARCH. II. THE ACTION OF AMYLASES ON RAW WHEAT STARCHES<sup>1</sup>

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Several factors are probably involved in effecting the differences in diastatic activity of flours in either suspensions or doughs. Among these factors might be (1) inherent differences in the starches from different wheat varieties, (2) effects of soil or climatic conditions during the growth of the grain, (3) size distribution of the starch granules, (4) readily available material such as broken starch granules, (5) types or (6) amount of enzymes present, (7) effects due to harvesting conditions including exposure of the grain in the field, and (8) storage conditions and processes such as steeping and bleaching during milling. Several or all of these factors are probably responsible for the variability in so called "diastatic activity" of flours, which ultimately involves the action of the amylolytic enzymes on the starch substrate.

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Many of the factors mentioned have been investigated. Spaeth (1915) concluded that weak flours have more small starch granules and that the quantity depends upon soil and climate conditions. Buchanan and Naudain (1923) found, however, that strong flours have the largest percentage of small granules, and that the average granule size is most important. Grewe and Bailey (1927) concluded that there is no correlation between granule size and the baking strength and diastatic activity of flours. Karacsonyi and Bailey (1930) and Malloch (1929) showed that overgrinding of flours results in increased diastatic activity. Ivanov, Kurgatnikov, and Kirsanova (1937) found that starch from peas grown in a hot dry climate was hydrolyzed much more slowly than starch from peas grown in a moist climate. Mangels (1926) found raw durum starch more easily attacked by diastase than raw spring wheat starch, and he later (1932) observed that starch from bleached flour was more easily hydrolyzed by taka-diastase than starch from unbleached flour. Sandstedt, Blish, Mecham, and Bode (1937) concluded that with the possible exception of durum wheat flour the unruptured granules of various flours are for all practical purposes identical in their resistance to attack by enzymes.

Although alpha-amylase has generally been found to increase the diastatic activity of flours and the action on raw wheat starches, Blish, Sandstedt, and Mecham (1937) stated that normal wheat flour contains an enzyme factor capable of accelerating the saccharification of raw starch, but that the most active preparation of this raw starch factor can be obtained from malted wheat flour. They also described a non-enzymic inhibitor and a non-enzymic "activator" of the raw starch factor. The latter was thought to function only by counter-acting the inhibitor.

### Experimental

*Preparation of enzymes.*—Two preparations of alpha-amylase and two of beta-amylase were made from Thatcher wheat. The beta-amylase preparations were from normal wheat. For the alpha-amylase preparations the wheat was steeped for two days at about 16° C. and then allowed to germinate at 20° C. for three days, after which it was dried at 25° C. One method used in preparing the enzymes was that described by Klinkenberg (1932) by which alpha-amylase is precipitated by 60% alcohol and beta-amylase by 80% alcohol from the respective extracts. The second method was a combination of Ohlsson's (1926) and Klinkenberg's methods. According to Ohlsson the alpha-amylase extract is heated to 70° C. for 15 minutes, thus inactivating most of the beta-amylase present, and the

beta-amylase extract is acidified with hydrochloric acid to pH 3.3 for 15 minutes at 0° C., a treatment which inactivates most of the alpha-amylase. The method employed was to treat the extracts according to Ohlsson's technique and then precipitate the enzymes by Klinkenberg's method. The enzyme preparations made by Klinkenberg's method will be referred to as -1 and those prepared by the combined procedures of Ohlsson and Klinkenberg as -2. A commercial diastase preparation (Merck) was also used and will be referred to as malt diastase.

*Action of enzymes on soluble starch.*—Before using the enzyme preparations for raw-starch hydrolysis some tests were carried out with Lintner soluble-starch (Merck) solutions as substrates in order to characterize the preparations as to their relative dextrinizing and saccharifying activities.

Wohlgemuth (1908) tests for the dextrinizing action were made by using 3 c.c. of 2% boiled soluble-starch solution buffered at pH 5.1 and 3 c.c. of water and enzyme solution, with hydrolysis for one hour at 40° C. The values as expressed in Table I are in terms of milligrams

TABLE I

RESULTS OF WOHLGEMUTH, CALDWELL-HILDEBRAND, AND SACCHARIFYING TESTS OF THE ENZYME PREPARATIONS, WITH SOLUBLE STARCH AS SUBSTRATE

Enzyme	Wohlgemuth value, mgs. starch per mg. enzyme	Erythro-R value, % hydrolysis	Starch dextrinized but not precipitated in 55% alcohol	Percent hydrolysis, from reducing-sugar determinations (as maltose)		
				In 5 hrs.	In 12 hrs.	In 28 hrs.
	mgs.	%	%	%	%	%
Alpha-amylase-2	214	15.3	64.4	49.5	49.5	49.5
Alpha-amylase-1	171	37.8	46.4	80.0	84.5	89.0
Malt diastase	100	44.4	40.4	78.1	84.5	87.1
Beta-amylase-1	<1	—	7.4	61.0	61.0	63.7
Beta-amylase-2	<1	—	3.7	31.4	52.0	61.0

of starch hydrolyzed to the red-violet (erythro) end point per milligram of enzyme preparation. The reducing values at the end points (the erythro-R values) in terms of percent hydrolysis determined as maltose are also given in Table I.

The dextrinizing activity of the enzymes was determined according to the method described by Caldwell and Hildebrand (1935). A 25 c.c. portion of 2% soluble-starch paste (500 mg.) buffered at pH 5.1 was hydrolyzed for 10 minutes at 30° C. with 10 mg. of enzyme. Ethyl alcohol was then added to give a concentration of 55% by volume. The precipitate was first washed with 55% alcohol and then with 95% alcohol, after which it was dried at 105° C. and weighed.

The percentage of starch dextrinized and not precipitable in 55% alcohol is given for each enzyme preparation in Table I. A higher value indicates higher dextrinizing activity of the enzyme preparation.

The saccharifying activity of the enzyme preparations was compared by using 25 milligrams of enzyme in 100 c.c. of 1% soluble-starch solution buffered at pH 5.1 with hydrolysis at 30° C. Reducing-sugar determinations (calculated as maltose) were made by the ferricyanide reduction method (Blish and Sandstedt, 1933) and Table I gives the percent hydrolysis of the starch after 5, 12, and 28 hours, respectively.

Wijsman (Klinkenberg, 1932) tests were made by placing drops of enzyme solution on a sterile gelatin and starch medium in petri dishes

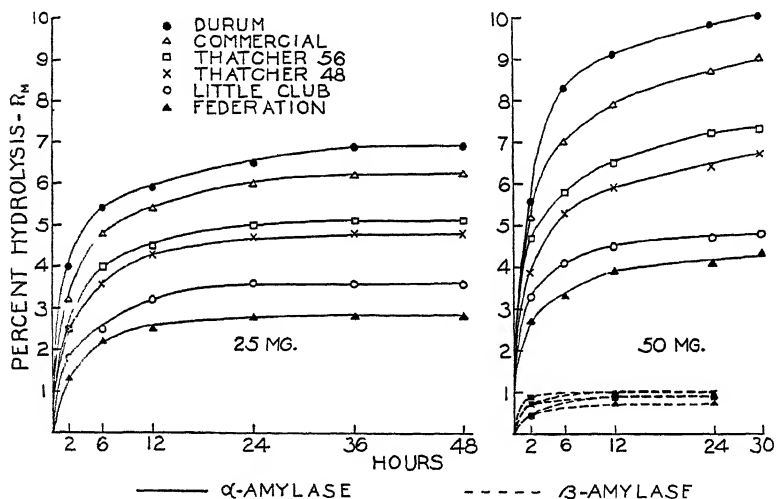


Fig. 1. Action of 25 and 50 mg. of alpha-2, and 50 mg. of beta-2 on raw wheat starches.

which were incubated for four days at 5° C. and then stained with iodine. A colorless spot indicates alpha-amylase and a violet spot beta-amylase, while mixtures of the enzymes produce a colorless spot surrounded by a violet ring. The results indicated that malt diastase and alpha-amylase-1 were mixtures of the two enzymes, and that alpha-2, beta-1, and beta-2 were fairly pure preparations of their respective enzymes.

*Action of the enzymes on raw wheat starches.*—With the various wheat starches described in Part I available, namely from durum, Thatcher of 48 and 56 pounds weight, Little Club, Federation, and a commercial wheat starch, it was of interest to study the action of the amylases on the raw starches. Small starch granules about 3 to 6 $\mu$

in diameter and large starch granules about 20 to 35 $\mu$  in diameter, separated from Thatcher 56 and from the commercial wheat starch, were also used as substrates. Details as to the method used in separating the granules are given in Part I, which also includes a photomicrograph of the small and the large granules from Thatcher 56. All hydrolysates were 100 c.c. of 1% starch suspensions buffered with citrate solution at pH 5.1 and the temperature was maintained at 30° C. in all cases. Stoppered flasks were used and a drop of toluol was added to prevent bacterial action. A mechanical shaking device was used to keep the starch in suspension. The rate of hydrolysis was followed by determining the reducing value computed as maltose and expressed as percent hydrolysis of the starch present.

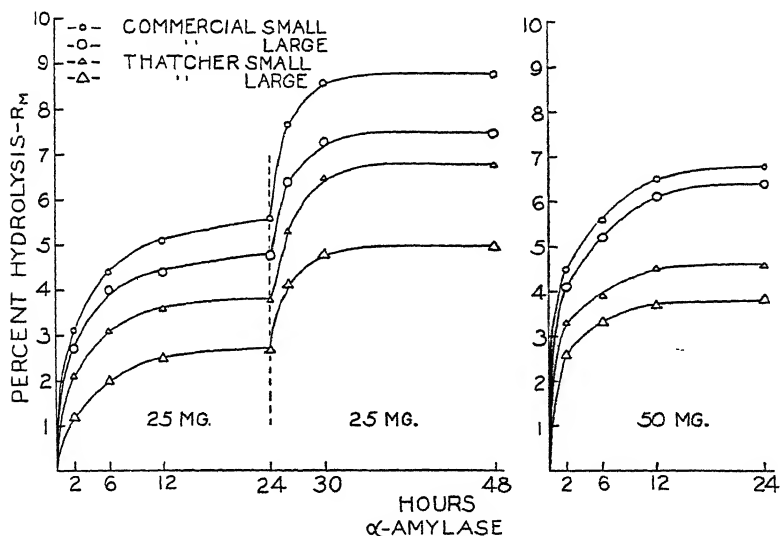


Fig 2. Action of alpha-2 on the small- and the large-granule fractions.

In the first series of tests 25 mg. and 50 mg. of alpha-2 and 50 mg. of beta-2 were allowed to act upon the raw starches. The hydrolysis was continued for 48 hours when 25 mg. were used, and for 30 hours when 50 mg. were used. The progress of the hydrolysis is shown graphically in Figure 1.

The action of alpha-2 on the small- and the large-granule fractions from Thatcher 56 and commercial starches is shown in Figure 2. In one case two 25-mg. portions were added, and in the other case 50 mg. were added directly.

Since the various starches exhibited differences in their susceptibility to the action of alpha-amylase, as shown by Figure 1, it was of interest to ascertain if the same relative susceptibility remained after

the starch granules had been thoroughly disintegrated. For this purpose, starch samples were used which had been pulverized in the rod mill by grinding for 84 hours as described in Part I. The amount of enzyme used was 25 mg. of alpha-2 and 50 mg. of beta-2. The graphs in Figure 3 show the results of 24 hours of hydrolysis. The action of beta-1 was found to be practically the same as that of beta-2, and only about 1.1% of the raw starches was hydrolyzed during 24 hours.

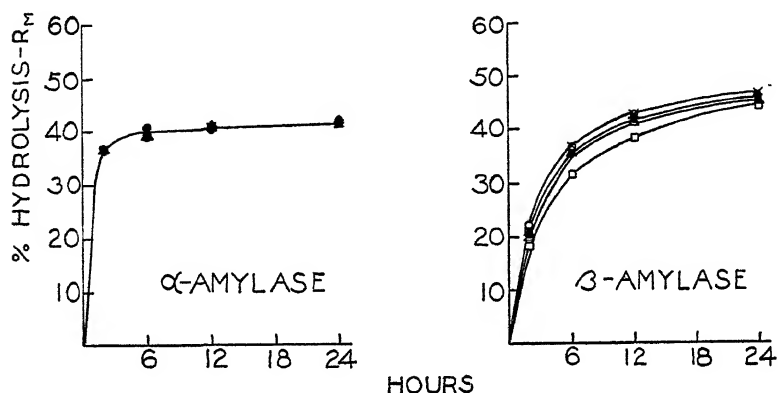


Fig. 3. Action of alpha-2 and beta-2 on the pulverized raw wheat starches. In the case of alpha-amylose, all six starches gave practically the same results and hence only one line was drawn while with beta-amylose a slight variation was noticed.

The hydrolysis of the raw starches by alpha-1, malt diastase, and alpha-2 was then compared. Durum, Federation, and Thatcher 56 starches were used as substrates. The amount of enzyme used was 50 mg. and the hydrolysis continued for 24 hours. In the instance of alpha-1 an additional amount of enzyme equivalent to 50 mg. was added after 24 hours and the hydrolysis continued up to 36 hours. The data obtained are shown by the graphs in Figure 4.

It is evident from Figure 4 that alpha-1 was more active on the raw starches than alpha-2, although the data in Table I show that alpha-2 was the more active dextrinizing enzyme. This suggests the possibility that the greater action of alpha-1 on the raw starches was due to the presence of appreciable amounts of beta-amylose. To study this possible effect of mixtures of the amylases on the raw starches a series of tests was made with alpha-2 and with beta-2 alone and in various combinations. The substrate was raw Thatcher 56 wheat starch. The data presented by the graphs in Figure 5 show the results of these tests and also rate of hydrolysis of alpha-1, so that a direct comparison can conveniently be made.

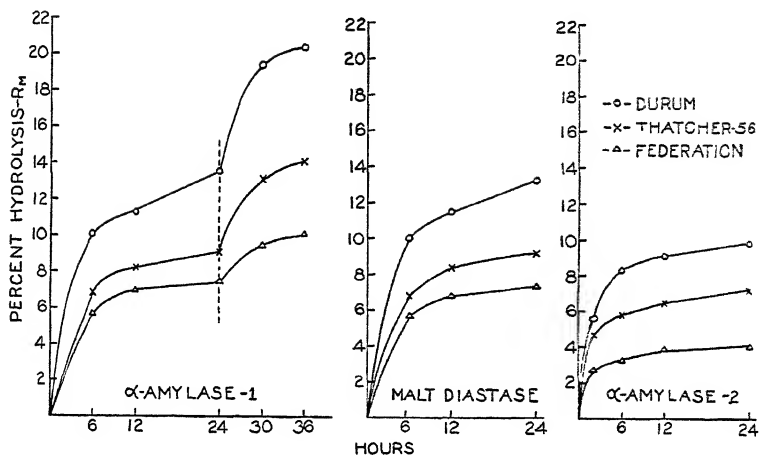


Fig. 4. Action of 50 mg. of alpha-1, malt diastase, and alpha-2 on raw wheat starches. An equivalent amount of alpha-1 was added after 24 hours.

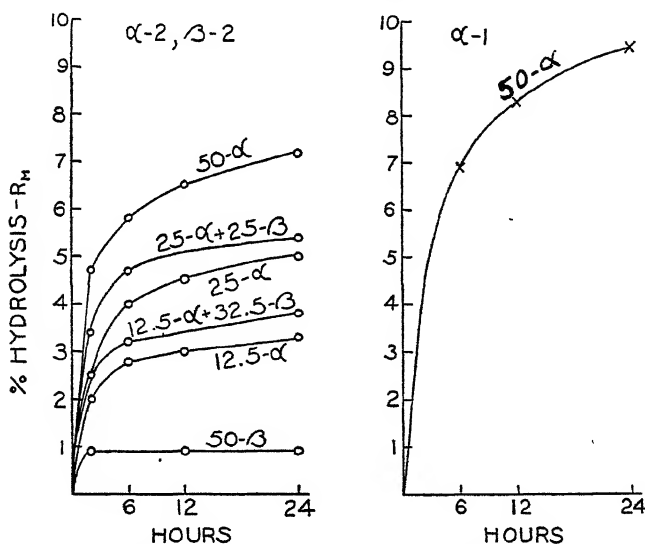


Fig. 5. Action of alpha-2 and beta-2 and various mixtures of them as compared to alpha-1. The milligrams of each enzyme are indicated on the graphs.

### Discussion

The results indicate that beta-amylase will not hydrolyze raw starch to a great extent. Only about 1% of the starch was hydrolyzed by the beta-amylase preparations.

The action of the alpha-amylase preparation indicates differences in enzyme susceptibility of the raw starches as shown by Figures 1 and 4. If the observed differences were due to varying amounts of

readily available material such as broken granules, the progress curves with beta-amylase should show such differences, since beta-amylase readily attacks the pulverized granules as shown in Figure 3. Thus it appears quite probable that differences in alpha-amylase susceptibility really existed. The durum starch was most easily hydrolyzed, which is in agreement with Sandstedt, Blish, Mecham, and Bode (1937) and Mangels (1926, 1932). Next were commercial, Thatcher 56, Thatcher 48, Little Club, and Federation in order of increasing resistance to hydrolysis.

The small granules showed slightly less resistance to hydrolysis than the large granules. The fractions from commercial starch were hydrolyzed more readily than the fractions from Thatcher 56, which is in agreement with the original starches as shown in Figure 1. Neither the small nor the large granule fractions were hydrolyzed as readily as their respective original starch samples, suggesting that the additional water treatment necessary to separate the large and the small granules may have removed the more labile material.

Since the small granules were more easily hydrolyzed than the large granules, it seems possible that the observed differences in the enzyme susceptibility of the six starch samples might be due to granule-size variations. A careful analysis of the graphs in Figures 1 and 2 shows that the differences in enzyme susceptibility of the six starch samples were much greater than those of the small and the large granules, and hence it is reasonable to conclude that the differences were not due to the granule-size distribution. This appears to be in agreement with Grewe and Bailey (1927) that there was no correlation between starch-granule size and diastatic activity of flours.

Figure 3 shows that the raw-starch granules which had been pulverized in the rod mill were as easily hydrolyzed by both alpha- and beta-amylase as boiled soluble-starch solutions. Taylor and Keresztesy (1936) observed that dry grinding or Lintner acid treatment of corn starch produced similar changes when the alkali lability was used as a criterion. Alsberg and Griffing (1925), and Karacsonyi and Bailey (1930) noticed that overgrinding of flour resulted in substantial increase in the diastatic activity.

The enzyme hydrolysis of the ground raw starches further indicates that after the granule structure has been thoroughly broken down the various starches show practically no difference in the rate of enzyme hydrolysis. Thus it appears that the observed variations in enzyme susceptibility of the raw starch granules are due primarily to differences in the granule structure. Probably the porosity of the granule is a factor. Lynst-Zwikker (1921) studied the action of the

amylases on potato and wheat starches and suggested that the amylophosphoric ester in potato starch is combined with potassium and in wheat starch with calcium, and that the calcium ion makes the wheat starch more permeable for the amylases and hence contributes to the greater enzyme susceptibility of wheat starch as compared to potato starch. Samec (1934) also suggested that calcium ions are present in wheat starch and potassium ions in potato starch, but he did not discuss this in relation to enzyme susceptibility.

The conclusion reached in Part I, that there was no significant difference in the relative amounts of amylopectin in the various wheat starches, indicates that the amylopectin content is not the factor involved in the observed variability in resistance to enzyme hydrolysis of the wheat starches. Lynst-Zwicker (1921) reached the same conclusions. On the contrary, Weichsel (1936) concluded that tuber starches such as potato starch differ from wheat starch by having a higher amylopectin content and that this is responsible for the higher resistance of potato starch to enzyme action. She determined the amylopectin content by washing and centrifuging triturated starch and called the insoluble portion amylopectin. Most investigators, however, find less amylopectin in potato starch. Taylor and Iddles (1926) obtained 1.8% from potato starch and Taylor and Walton (1929), using the same electrophoretic technique, reported 25.5% in wheat starch.

The phosphorus content of the various starches is given in Part I, and there appears to be no correlation between the phosphorus content of the wheat starches and their enzyme susceptibility in the raw state.

It seems possible that the small differences exhibited by various types of raw wheat starches are due mainly to morphological differences of the granules as a result of inherent genetic qualities and to environmental factors during growth of the grain.

The actions of alpha-1 and malt diastase on the raw wheat starches were very similar, and both enzymes hydrolyzed raw starch to a greater extent than alpha-2, which was the most active dextrinizing enzyme according to the data in Table I. The results obtained by the various amylase mixtures shown in Figure 5 indicate that the more vigorous action of alpha-1 (and malt diastase) on the raw starches was probably not due to the presence of beta-amylase in appreciable amounts. Although alpha-2 was the most active dextrinizing enzyme with soluble starch as substrate, this enzyme preparation probably contained less of the "raw starch factor" described by Blish, Sandstedt and Mecham (1937).

Figure 4 shows that, upon the addition of a second portion of alpha-1 after 24 hours of hydrolysis, the same relative susceptibility



of the starches was observed. The durum starch was most easily hydrolyzed by both portions of enzyme, followed by Thatcher 56, with Federation starch being most resistant to hydrolysis. This is noticed also by the second portion of enzyme added to the small and the large granule fractions as shown in Figure 2. This indicates that the starch granules are quite uniform in structure throughout.

### Summary

Two alpha-amylase preparations were made from germinated wheat, the first by direct alcohol precipitation and the second by a heat treatment of the extract followed by alcohol precipitation; they are referred to as alpha-1 and alpha-2 respectively. Two beta-amylase preparations were made from normal wheat, the first by direct alcohol precipitation, the second by an acid treatment of the extract followed by alcohol precipitation, and are referred to as beta-1 and beta-2, respectively. A commercial malt diastase preparation was also used. These enzyme preparations were first compared by several methods as to their relative dextrinizing and saccharifying activity with soluble starch solutions as substrates.

Beta-amylase hydrolyzed only about 1% of the raw wheat starch granules. Alpha-amylase hydrolyzed raw wheat starches to a greater extent, about 4 to 10% with the amounts of enzymes used, and differences in alpha-amylase susceptibility of the raw starches were noticed. Durum starch was most readily hydrolyzed, followed by the commercial wheat starch, Thatcher 56, Thatcher 48, and Little Club, while Federation starch was most resistant to hydrolysis.

Alpha-amylase hydrolyzed the small granules more readily than large granules in the raw state, but the differences were smaller than those observed for the various starch samples. Hence it was concluded that the differences in enzyme susceptibility of the raw starches were not due to granule size. Neither did the differences appear to be due to more or less easily hydrolyzed material such as broken granules.

Raw wheat starches which had been finely pulverized by grinding for 84 hours in a rod mill were easily hydrolyzed by both beta- and alpha-amylase and with no significant degree of difference in the various starch samples. Such pulverized granules in the raw state were about as easily hydrolyzed as soluble starch paste.

There was no correlation between the phosphorus content of the wheat starches, as given in Table III, Part I, and the enzyme susceptibility of the raw starches.

It was suggested that the small differences exhibited by various types of raw wheat starches are due mainly to morphological differences of the granules.

Alpha-amylase-2, which effected the greatest dextrinization of soluble-starch solutions, was less active on raw wheat starches than alpha-amylase-1 and malt diastase. This was apparently not due to the presence of beta-amylase in the last two enzyme preparations, but it is suggested that possibly alpha-amylase-2 contained less of the "raw starch factor" than the other enzyme preparations.

The rate of hydrolysis of the raw wheat starch granules after successive increments of enzyme were added indicated that the same degree of resistance was present throughout the granule.

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### STUDIES ON WHEAT STARCH. III. THE ACTION OF AMYLASES ON WHEAT AMYLOPECTIN AND AMYLOSE<sup>1</sup>

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Many investigators have found that a solution of amylose will turn turbid upon standing with the formation of "retrograded amylose," which is considered a less hydrated state of amylose. Katz and Itallie (1931) found that amylopectin and amylose separated by electrophoresis from autoclaved potato starch showed retrogradation, as determined by the X-ray method. The solutions were kept for one to two months at 2°-3° C. The V spectrum of the original fractions changed to a B spectrum upon standing. They did not agree with statements in the literature that amylopectin does not retrograde. The authors did not, however, state the completeness of separation of the amyloses and a small amount of amylose in the amylopectin fraction would probably result in a change in X-ray spectrum.

As to the physical properties of the two fractions it is generally agreed that amylopectin is least soluble and forms a more viscous turbid solution which gives a red-violet color with iodine. Amylose is most soluble and least viscous and gives an almost clear solution which becomes a brilliant blue on treatment with iodine. A solution of amylose will retrograde upon standing and turn turbid, finally depositing a white precipitate. The rate of the retrogradation depends on the concentration of the solution and the temperature.

The degradations, particularly by enzymes, of the amylopectin and amylose have led to many theories as to the structure of the fractions and the degradation products. From the physical state of

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the fractions, it might be expected that the more soluble amylose fraction would be more easily hydrolyzed by enzymes. Sherman and Baker (1916) effected a separation of a 20% starch paste containing 0.002 M sodium chloride by using the centrifuge and calling the soluble fraction amylose. With fractions thus separated they found the amylose to be slightly more easily hydrolyzed by malt diastase than the amylopectin fraction. Sjöberg and Eriksson (1924) separated the fractions from potato starch by the alkali leaching method and found the amylose to be more readily hydrolyzed by amylases from both normal and germinated barley. Ling and Nanji (1925) studied the action of barley diastase on the starch fractions separated by the freezing methods and found amylose to be most readily hydrolyzed. They concluded that the difference between amylose and amylopectin is mainly steric, with the former being aggregates of alpha-hexa-amylose and the latter of alpha-beta-hexa-amylose. Josephson (1927) discussed a hypothesis that the amyloses are aggregates of elementary molecules, these units being anhydro-disaccharide for amylose, and for amylopectin a complex of the anhydro-disaccharide plus anhydroglucose.

Polak and Tychowski (1929) studied the action of amylases on amylopectin and amylose separated from potato starch by the Sherman and Baker centrifuge method. They concluded that amylopectin is hydrolyzed by alpha-amylase to give dextrin I, which has six maltose units, and that amylose is hydrolyzed by beta-amylase to maltose but by alpha-amylase to dextrin II with three maltose units. Pringsheim (1932) believed amylose is degraded to produce dihexosans and amylopectin to trihexosans, but Myrbäck and Ahlborg (1937) considered the presence of such units contrary to known facts from studies of the limit dextrans.

Samec and Waldschmidt-Leitz (1931) separated amyloamylose and erythroamylose from potato starch by electrophoresis. The amyloamylose was almost completely hydrolyzed by malt and pancreatic amylase. The erythroamylose was hydrolyzed to the extent of 70%, 96%, and 56% by pancreatic, malt, and barley amylases, respectively. Freeman and Hopkins (1936) obtained 20% amyloamylose from autoclaved potato starch by electrophoresis. This was hydrolyzed to the extent of 96.8% by barley amylase after 48 hours, while the erythroamylose fraction was only 57.7% hydrolyzed.

It appears that the previous work has been almost exclusively with amylopectin and amylose from potato starch, separated by different techniques giving fractions which probably varied considerably in completeness of separation and in properties. Hence a study of the action of the wheat amylases on wheat amylopectin and amylose was made, with separation by the electrophoretic technique.

### Experimental

Many workers have failed to state the completeness of separation of the amyloses used for enzyme substrate, and in the majority of cases boiled starch solutions were used and the amylopectin fraction undoubtedly contained many entire, unruptured granules.

The fractions used as substrates in this study were prepared from commercial wheat starch which had been thoroughly pulverized by grinding in a rod mill for 84 hours. The ground starch was then fractionated by the electrophoretic method as described in Part I. The amylopectin used represented 17% of the starch and contained 0.261% phosphorus, which was 73.8% of the total phosphorus of the original starch from which it was separated. The amylose preparation represented about 80% of the total amylose obtained from the starch and contained 0.001% phosphorus. The amylases used were the purer fractions, alpha-amylase-2 and beta-amylase-2, prepared from wheat as described in Part II.

A series of hydrolysates was made, using freshly prepared substrates, and the hydrolysis was at 30° C. buffered to pH 5.1 with a citrate solution. In all instances 25 c.c. of 1% substrate and 12.5 mg. of total enzyme (same as 50 mg. per 100 c.c. of 1% substrate) or the equivalent was used. Hydrolysates with alpha-amylase alone, beta-amylase alone, and alpha- and beta-amylase combined were allowed to act for 24 hours. An equivalent amount of enzyme was then added and the hydrolysis continued up to 44 hours. The rate of hydrolysis was determined by the reducing-sugar method and expressed as percent hydrolysis in terms of the theoretically available maltose. The results are shown in Figure 1.

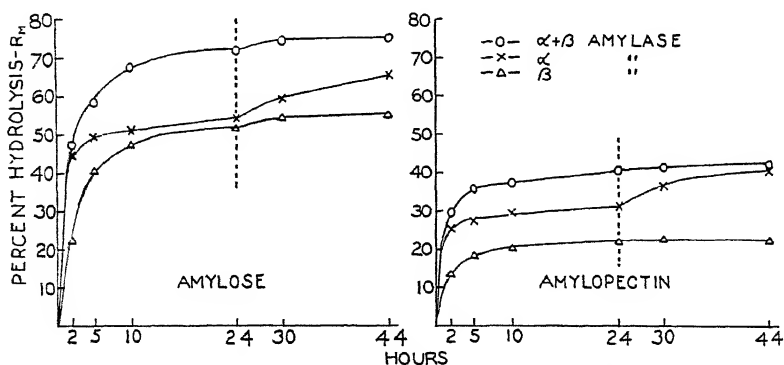


Fig. 1. Action of alpha- and beta-amylases and their mixtures on amylopectin and amylose. An additional equivalent quantity of enzyme was added after 24 hours' hydrolysis.

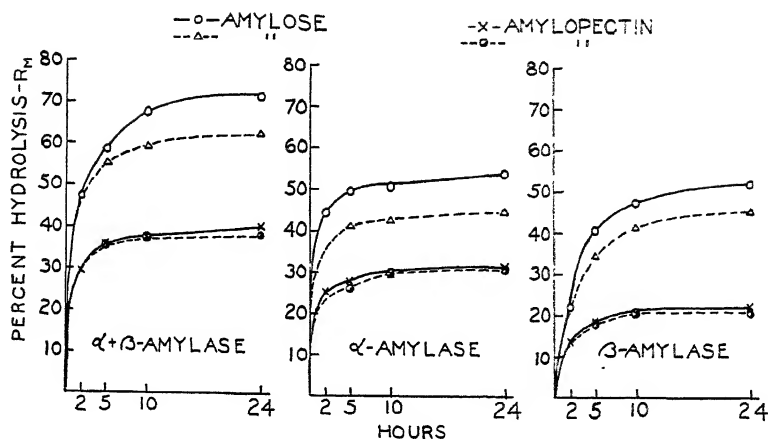


Fig. 2. The effect of retrogradation. The solid lines show the action of the amylases on freshly prepared substrates, the dotted lines are substrates which had been stored as 2% solutions for 2 days at 5°. The amylose and amylopectin were from commercial wheat starch.

It was noticed that when 2% amylose solution was allowed to stand at 5° C. it became turbid in about a day with the formation of a white precipitate. At room temperature this retrogradation required about three weeks, using a 2% solution. A study of the effect of retrogradation on enzyme susceptibility was made using both amylose and amylopectin.

TABLE I

ACTION OF BETA-AMYLASE ON AMYLOSE SOLUTIONS FRESHLY PREPARED AND AFTER STORAGE FOR 2 AND 8 DAYS AT 5° C.—HYDROLYSIS AT 30°, PH 5.1—ALL VALUES IN PERCENT HYDROLYSIS- $R_M$

Period of hydrolysis	Freshly prepared amylose solution	After 2 days' storage	After 8 days' storage
<i>Hours</i>	<i>%</i>	<i>%</i>	<i>%</i>
2	27.8	25.5	24.7
6	43.2	37.5	35.5
12	44.7	40.4	—
24	46.6	41.2	38.8

The rates of hydrolysis of freshly prepared solutions were determined, and 2% solutions of amylopectin and amylose, with a drop of toluol to prevent bacterial activity, were allowed to stand for two days at 5° C. and the enzyme hydrolysis was again studied. The same temperature, pH, substrate, and enzyme concentrations were used. The results are shown in Figure 2.

Amylose was precipitated by addition of alcohol to aliquots of a solution, dried, and ground to a fine powder. A few weeks later a 2% solution was made and used as enzyme substrate, freshly prepared and also after storing for 2 and 8 days respectively. Beta-amylase only was used in this instance, and the conditions were the same as for the previous tests. The results are recorded in Table I.

### Discussion

The amylopectin was found to be much more resistant to the action of the amylases alone or in combination, than the amylose, and the amylopectin was more resistant to the action of beta-amylase than alpha-amylase. Combinations of alpha- and beta-amylase hydrolyzed the amylopectin slightly more rapidly than alpha-amylase alone. After 24 hours' hydrolysis, 22% of the amylopectin was hydrolyzed by beta, 31.4% by alpha, and 40.2% by alpha- and beta-amylase combined. The addition of more enzyme did not produce much further hydrolysis except with alpha-amylase, which effected 40.8% hydrolysis after 44 hours.

After 24 hours 51.8% of the amylose was hydrolyzed by beta, 54.1% by alpha, and 71.4% by alpha- and beta-amylase combined. Addition of more enzyme increased the hydrolysis by alpha to 65.6% after 44 hours, with only slight increases on like additions of enzyme to the other hydrolysates. The amylose from wheat starch was not completely hydrolyzed as was observed with amyloamylose from potato starch by Samec and Waldschmidt-Leitz (1931) and Freeman and Hopkins (1936), but the amyloamylose in the first case represented only about 50%, and in the latter case only 20% of the starch. The amylose used in this study was 83% of the wheat starch. Thus the incongruity in results is probably due to different methods of separation and different starch fractions used, as well as variations in the enzyme preparations and conditions of hydrolysis.

Retrogradation of amylose increases the resistance to the hydrolyzing action of beta- and alpha-amylases and their mixtures, as shown in Figure 2. The data in Table I indicate that most of the retrogradation took place during the first two days, and with only a slight additional change between the second and the eighth day of storage at 5° C. The storage of the 2% solution of amylopectin for two days at 5° C. produced very little change, as indicated by the rate of enzyme hydrolysis.

It is possible that wheat amylopectin and amylose differ from those separated from potato starch, in view of the theories of Samec (1934) and Koets (1935), that wheat starch contains a nitrogenous material not present in potato starch.

### Summary

Amylose from wheat starch was much more easily hydrolyzed by both alpha- and beta-amylases and combinations of them than the amylopectin fraction.

Amylopectin was hydrolyzed by alpha-amylase more readily than by beta-amylase.

Amylose solutions which were allowed to retrograde for two days at 5° C. were more resistant to hydrolysis by both the amylases and their combination than was the freshly prepared amylose. Amylopectin solutions showed very little change in enzyme susceptibility after a similar storage treatment.

The decrease in enzyme susceptibility of the amylose solution was most rapid within the first two days of retrogradation at 5° C.

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# THE USE OF DIELECTRIC MEASUREMENTS TO DETERMINE THE MOISTURE CONTENT OF POWDERY SUBSTANCES

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Very many food products such as flour, granulated sugar, starch, meal, grain, and others are powdery or friable substances. Besides, many products like biscuits, lump sugar, rusks and others can easily be converted into powders. Moisture determinations on such products are frequently made in food laboratories, and this circumstance has led us to choose these products as materials for research work in the determination of moisture content by the dielectric constant method.

Many investigators have studied the problem of determining the dielectric constant (DK) of powdery substances. Thwing (1894) was the first to determine the DK of powdery substances in a mixture with alcohol or ether. He made calculations according to the rule of mixtures. Starke (1897) employed isoelectric liquids and obtained good results for substances which are non-conductors of electricity. Loewe (1898) should be mentioned among other investigators and in particular J. Tausz and Rumm (1934), who carried on extensive work in the study of the DK of certain powdery and fibrous substances. These investigators, however, did not assume the task of using DK measurements for determining moisture content. While some of them pointed out such a possibility, no investigations on this problem were made.

Berliner and Rüter (1929) were the first to use DK measurements for a practical purpose, and they recommended that the moisture content of flour and grain be determined by the DK method. They obtained results which indicated that DK measurements were wholly applicable for the determination of moisture content with sufficient accuracy for practical purposes.

Other investigators confirmed their findings. This, during the past five years, has led to the appearance of many dielectric-measurement instruments for the determination of moisture content. While these instruments made for very rapid and technically accurate determinations of moisture content, they had the shortcoming of being too specialized, that is, of being suitable only for the one substance for which their scales were calibrated. No general methods for determining the DK and therefore the moisture content of any powder have as yet been elaborated.

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The principle underlying the determination of a DK which is due in part to moisture is not different from that underlying the determination of the DK of ordinary dielectrics. The dielectric constant of dry substances classified as food products attains a magnitude of not more than 8 to 10, while the DK of water approximates 80. It follows from this that a change in the composition of dry substances affects the DK of the entire substance very little, while a slight increase or diminution of the moisture content immediately raises or lowers its DK. This statement does not hold for substances containing colloids and consequently able to combine with water, with a resultant large reduction of the DK of the water in combination. In cases therefore in which the moisture content is slight, *i.e.*, when the water is mostly held in combination, the DK change with variable moisture content is very slight and quite out of proportion to the DK of water. This greatly complicates the use of the dielectric method. This complication is also met of course in dealing with our powdery substances, but there are other specific difficulties which must be overcome if the purpose of obtaining satisfactory estimation of moisture is to be attained.

The following method of determining the moisture content of powders by DK can easily be imagined schematically. A test powder is poured into a condenser having a certain electrical capacity. This condenser is connected to an instrument, and the increase of its capacitance is determined. The moisture content sought is calculated according to a previously drawn curve showing the degree to which the DK of the powder or the capacitance of the condenser is dependent on the moisture content of the powder.

The entire measurement would require literally one minute were it not for the following complications. First, the DK of substances which contain water depends greatly on the temperature and hence the temperature must necessarily either be held constant or else corrections must be made for its changes. Second, the powder is not a homogeneous substance, like a liquid, but is a mixture of the powder substance itself and air. The dielectric constant of such a two-component dielectric will depend on the ratio of the component parts and consequently in any given case on the magnitude of compression and the size of the powder particles.

The first difficulty can easily be overcome. The second, however, is one of the chief obstacles standing in the way of determining with satisfactory accuracy the moisture content of powders. Before beginning to work out a method suitable for determining the moisture content of any powder, it is first necessary to devise a method of eliminating the effect which variations in compression have on the results.

The simplest solution, it seems, would be to have identical compression when the powder is poured into the condenser. But this is extremely difficult to attain. There are two ways: either to pour at all times without compression or to apply a definite degree of compression which has at all times the same magnitude (Henriquez and Renaud, 1935). In both cases special devices are required and this complicates the entire instrument. Moreover, the use of such devices is not feasible, because difficulties are encountered in that the degree of crumbling (*i.e.*, the size of the particles) affects the density and the ratio of the volumes which the substance and the air occupy. Hence identical compression cannot be obtained.

Wholly satisfactory results can be obtained only when crumbling is identical, a condition which is very seldom met with in practice; however, in most cases the degree of compression can be determined mathematically.

The problem can be approached from two angles. The powder can be regarded as a mixture of two elements—the powder substance itself and air. With the DK of such a mixture and the ratio of the volumes of the components, the dielectric constant of the powder substance can be calculated by a special formula. Or one can assume the powder to be a homogeneous dielectric and make no calculation of the DK of the substance, corrections for the degree of compression being made directly in the DK of the powder. There are many formulas by which the DK of the substance may be calculated from the DK of the mixture. They were worked out on the basis of the rule of mixtures. Examples are those of Lorenz-Lorentz, Beer, Newton, Silberstein, and Lichtenecker. See Lichtenecker (1926) and Brugge-man (1936).

Only the Lichtenecker formula has proved theoretically suitable for our purpose, since the use of the others is limited by many conditions (such as the form of the particles, the correlation of the parts, and so on). While Tausz and Rumm pointed out in their work (1934) that the formula of Lichtenecker gives good results for powders with particles of arbitrary form, they did not confirm this conclusion with sufficient experimental material. The following equation expresses the logarithmic rule of mixtures on which the formula of Lichtenecker is based.

$$E_m = E_1^{\delta_1} \cdot E_2^{\delta_2}$$

where  $E$  is the DK of the mixture and  $E_1$  and  $E_2$  are the DK values of the component parts, and  $\delta_1$  and  $\delta_2$  are fractions of the volume occupied by the parts. If one substance is air, having a DK almost equal to unity, and if  $E_2$  is taken as the DK of air, then

$$E_m = E_1^{\delta_1}$$

and

$$\log E_m = \delta_1 \log E_1$$

or

$$\log E_1 = \frac{\log E_m}{\delta_1} \quad (1)$$

Consequently, knowing the DK of the mixture and  $\delta_1$ , the fraction of the total volume which the substance itself occupies, one can use this formula to calculate the DK of a substance. The value of  $\delta_1$  can be determined if one knows the weight of the substance in a certain volume and its specific gravity, since

$$\delta = \frac{a}{vd}$$

where  $a$  is the weight of the substance,  $v$  is the volume occupied by the mixture (substance and air), and  $d$  is the specific gravity of the substance.

The same logarithmic rule can be used to make corrections for compression directly in the DK of the mixture. For this purpose we made the following transformation in the formula of Lichtenecker. In place of

$$\log E_m = \delta_1 \log E_1$$

one can write

$$\log E_m = \frac{a}{vd} \log E_1$$

where  $E_m$  is the dielectric constant of the mixture of the powder and air,  $a$  is the weight of the powder poured in the condenser,  $v$  is the volume of the condenser, and  $d$  is the specific gravity of the substance.

For the same powder, but where the degree of compression differs and consequently the weight also, the formula is

$$\log E_m^1 = \frac{a_1}{vd} \log E_1$$

where  $E_m^1$  is the new dielectric constant of the mixture of powder and air and  $a_1$  is the new weight of the powder poured into the condenser. On dividing one equation by the other one obtains:

$$\frac{\log E_m}{\log E_m^1} = \frac{a}{a_1}$$

and after solving for  $\log E_m$  one obtains

$$\log E_m = \frac{a \log E_m^1}{a_1} \quad (2)$$

If one knows the DK of a powder for one degree of compression ( $E_m^1$ ), this formula can be used to calculate the dielectric constant of the

powder as such ( $E_m$ ) for any degree of compression. The weight of the powder contained in the condenser characterizes the degree of compression.

Hence if any weight, 100 grams for instance, be taken as a comparative base, the results of the various tests can be recalculated and expressed as comparative results which would have been obtained had 100 grams of the material filled the condenser. Thus the influence of compression can be taken into account and comparative data obtained. Then the formula (2) takes the following form:

$$\log E_m = \frac{100 \log E_m^1}{a_1}$$

This formula has the advantage over the first (1) that it does not require that either the volume occupied by the substance or therefore the specific gravity of the substance be determined. Simplification of this nature results at the expense of generality since now only relative values reduced to the chosen weight of 100 grams are obtained. However, this formula yields results which differ only slightly from those obtained with the generalized Lichtenecker formula. This of course should be expected since the same logarithmic rule of mixture is fundamental to both formulas.

Since these results did not always prove satisfactory enough, we used the following formula, which turned out to be more suitable for the products investigated by us.

$$\log E_m = \frac{a^{\frac{1}{3}} \log E_m^1}{a_1^{\frac{1}{3}}}$$

or when  $a$  equals 100 grams,

$$\log E_m = \frac{100^{\frac{1}{3}} \log E_m^1}{a^{\frac{1}{3}}} = \frac{21.54 \log E_m^1}{a^{\frac{1}{3}}}$$

By using formulas to recalculate the findings in our work, we assumed the task of verifying experimentally the possibility of calculating the effect of compression on the DK of powders, and hence the possibility of determining their moisture contents accurately. Biscuits, flour, and granulated sugar served as research materials. The "Dielkometer," made by the Haardt firm, was used to measure the DK. The accuracy of the capacitance readings attained on this instrument is very great, reaching 0.001 cm. and less.<sup>2</sup> So from this viewpoint the instrument was very suitable for our purpose. Its shortcoming, however, is that it gives really precise measurement of DK only for

<sup>2</sup> A capacitance of 1 cm. = 10/9 micro microfarads.

dielectrics with slight electrical losses, that is, for non-conductors of electricity, such as many organic liquids—benzol, toluol, alcohol, or solid substances which only slightly absorb the electric vibrations.

But for substances with considerable losses the instrument gives more or less approximate values. This is due to the effect which the electrical loss has on the frequency of vibrations of the generator, which is the basic part of the instrument. On the Dielkometer, for example, we obtained the following data on the effect which the electrical conductivity of water and alcohol has on the resultant measurements of DK. The electric conductivity was varied by adding a one percent solution of potassium chloride in varying quantities. In the case of alcohol, the effect of the water added to the solution was also taken into account. Consequently the dependence on conductivity is very considerable.

<i>Liquid Used</i>	<i>DK</i>
Distilled water	80.00
100 c.c. H <sub>2</sub> O plus 0.04 c.c. 1% KCl	80.91
100 c.c. H <sub>2</sub> O plus 0.08 c.c. 1% KCl	82.24
100 c.c. H <sub>2</sub> O plus 0.12 c.c. 1% KCl	82.80
100 c.c. H <sub>2</sub> O plus 0.20 c.c. 1% KCl	81.54
100 c.c. H <sub>2</sub> O plus 0.24 c.c. 1% KCl	80.98

<i>Liquid Used</i>	<i>DK</i>
94 per cent alcohol	30.72
10 c.c. alcohol plus 0.04 c.c. 1% KCl	30.58
10 c.c. alcohol plus 0.28 c.c. 1% KCl	30.44
10 c.c. alcohol plus 1.2 c.c. 1% KCl	30.72
10 c.c. alcohol plus 1.8 c.c. 1% KCl	31.00
10 c.c. alcohol plus 4.0 c.c. 1% KCl	31.70

Graffunder and Weber (1931) present data indicating that the frequency of vibrations and therefore also the magnitude of the apparent DK of the substance depends on electrical conductivity. These authors propose a method of their own to eliminate this influence by attaching a device to compensate for the loss. The Dielkometer has no such device. Since the loss was fairly large in the products tested by us, rising with an increase in moisture content, we have to face the fact that what we have measured is not the true capacity but rather an approximate value.

The test powder was poured in a measuring condenser, made of two plates which were placed in a vessel having a mark near the top (Fig. 1). The vessel was fixed in an ebonite framework, making it possible to connect the condenser to the Dielkometer at all times in the same way. This is very important for precision measurements. The plates were 95 by 36 by 1.5 mm. in size and were placed 10 mm. apart. Up to the mark the vessel had a volume of 237 c.c. The empty condenser weighed 313.2 grams.

The Dielekometer is provided with two comparison condensers: a "grobkondensator" for coarse measurements, and a "feinkondensator" for precise measurements. In our work the correlation between the two comparison condensers was found, and the experimental condenser (Fig. 1) was then calibrated in terms of the "grob-

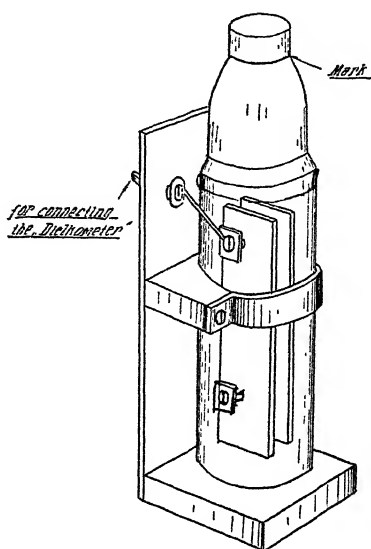


Fig. 1. Measuring condenser.

kondensator" alone, using mixtures of benzol and alcohol of known dielectric constants. The calibration curve is shown in Figure 2.

Instead of using the calibration curve as such, we found it more accurate to use its equation:

$$DK = 12.915 - 0.2186 n$$

where  $n$  is the reading of the comparative condensers (reduced to the "grobkondensator").

In order to avoid large fluctuations of temperature during the measurements the whole instrument was placed in a wooden case which was heated inside by electric lights. However, having in view the practical task of determining the moisture content by the DK and limiting ourselves by control measurements of the capacity of the empty condenser before the test, we did not aim to attain absolute constancy of temperature. The deviations from the indices, which were calculated when the calibrated curve was drawn up, gave corrections for temperature variations and for fluctuations in the voltage

of the battery. As the experiment showed, this was quite enough for practical work at a moderately fluctuating temperature ( $\pm 2^\circ\text{--}3^\circ$ ).

The powder was poured in up to the mark and slightly compressed (after which additional powder was poured in to fill up to the mark). The condenser was then connected to the instrument and a reading was taken, after which the full condenser was weighed on a technical balance having an accuracy of 0.1 gram. By deducting the weight of the empty condenser we obtained the weight of the powder.

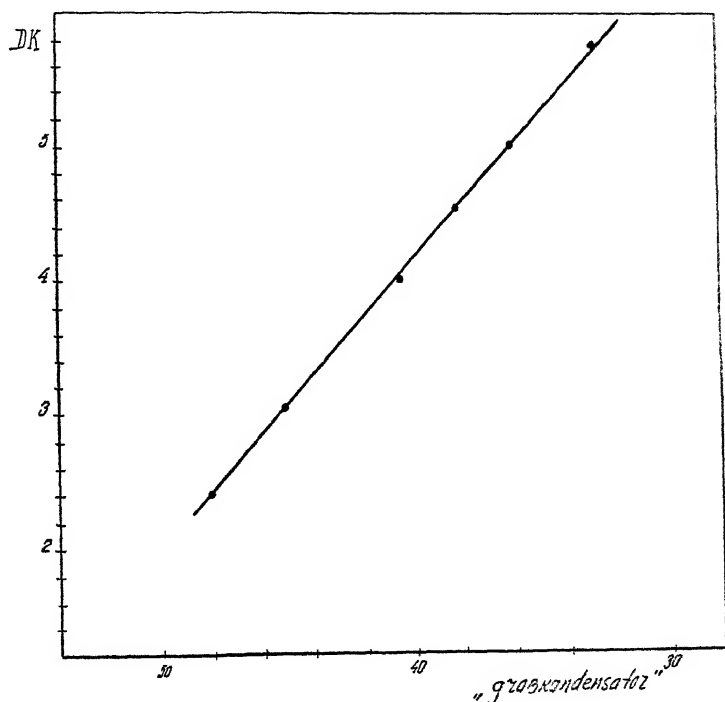


Fig. 2. Calibration curve.

By picnometric means in toluol we determined the specific gravity of the substance, which was essential in order to calculate the DK of the substance by the logarithmic formula. For each variety of material tested one common specific gravity was taken for samples of varied moisture content. The specific gravity, it should be noted, was somewhat reduced with an increase in moisture, but not enough to interfere with taking on average specific gravity for the entire series of tests.



The following powdery substances served as test materials:<sup>3</sup>

Ground biscuits, the sugary sort No. 1.

Ground biscuits, the sugary sort No. 2.

Ground biscuits, the sugary sort No. 2 (samples ground coarser than in previous case).

Ground biscuits, hard sort No. 3.

Wheat flour, 85% roughage.

Wheat flour, 79% roughage.

Granulated sugar.

A curve was first drawn for each of these substances showing the degree to which the dielectric properties depended on the moisture content. For this purpose we did as follows. The moisture content of the initial sample of each product was determined by the method employed in laboratories of the confectionary industry. Biscuits and flour were dried for 40 minutes at a temperature of 130 degrees. Granulated sugar was dried to a constant weight at a temperature of 100 to 105 degrees. The average test sample was poured in the measuring condenser up to the mark. The condenser was weighed and connected to the Dielkometer, and a reading of the capacity was taken on the comparative condenser. The pouring and readings were repeated five times.

After this, the initial sample was divided into two parts, one of which was placed in a water-containing desiccator for a certain length of time, resulting in a gradual increase in the moisture content of the substance. The other part was dried at a low temperature. After some time had elapsed and the substances had been stirred with great care, two samples of the same original product were obtained: one part having a moisture content greater and the other less than the initial sample.

These were tested in exactly the same way as the original samples. They were poured into the condenser, weighed, and a reading of capacity was taken. After this the samples with increased moisture contents were again made more moist, and those with lower moisture contents were dried further. Thus, two samples were again obtained, one having a still lower and the other a still greater moisture content. The results of measurements on the Dielkometer were recalculated by formula and an average was obtained for each sample. Thus the points of the curves were obtained.

<sup>3</sup> Recipes of dough of sugary sorts: 320 kg. of 30% wheat flour, 110 kg. powdered sugar, 0.3 kg. ammonium carbonate, 55 kg. butter, 2.2 kg. soda, 2.0 kg. salt, 2.0 kg. vanilla powdered sugar, 24 kg. eggs, 16 kg. starch, 40 kg. milk, 16 kg. lactins, 15 kg. invert sugar.

Recipes of dough of hard sorts: 320 kg. flour, 72 kg. powdered sugar, 32 kg. starch, 32 kg. butter, 2.8 kg. salt, 2.8 kg. soda, 0.6 kg. ammonium carbonate, 0.5 kg. vanilla powder, 12 kg. eggs, 90 liters milk, 6 kg. treacle.

To present all the calculations and results of the measurements in a given test would be too cumbersome. They number several hundred. Hence, we present in full but three examples, from which the sequence and essence of the tests are evident. Of the remaining experiments, however, only average figures will be presented. Table I shows the results of measurements for the dampened sample of Biscuit No. 1 with a moisture content of 10.76%. The same is given in Table II for the dried flour and in Table III for the granulated sugar.

TABLE I  
BISCUIT No. 1, MOISTURE CONTENT 10.76 PERCENT

Readings on "Grobkon- densator"	Readings on "Feinkon- densator"	Summary read- ings in terms of "Grobkon- densator" alone	DK of mixture of substance and air <sup>1</sup>	Weight of condenser with substance
1	2	3	4	5
46.05	50.0	46.05	2.848	432.5
46.05	51.5	46.10	2.838	432.9
46.05	53.9	46.19	2.818	431.3
46.05	57.8	46.32	2.790	429.3
46.05	48.0	45.97	2.866	432.3

Weight of substance	Specific gravity of substance	$\delta = a/vd$	DK of substance calculated by Lichtenecker formula	DK of mixture reduced to 100 g. according to our formula
6	7	8	9	10
119.3	1.346	0.374	16.45	2.523
119.7	1.346	0.375	16.14	2.522
118.1	1.346	0.370	16.45	2.526
116.7	1.346	0.366	16.50	2.522
119.1	1.346	0.373	16.82	2.522

<sup>1</sup> Calculated from the equation of the calibration curve (Fig. 2).

TABLE II  
FLOUR, MOISTURE CONTENT 9.0 PERCENT

Readings on "Grobkon- densator"	Readings on "Feinkon- densator"	Summary read- ings in terms of "Grobkon- densator" alone	DK of mixture of substance and air <sup>1</sup>	Weight of condenser with substance
1	2	3	4	5
46.8	50	46.8	2.685	448.4
46.8	48.4	46.74	2.698	447.9
46.8	52.8	46.90	2.663	445.9
46.8	52.3	46.88	2.667	446.6
46.8	51.9	46.87	2.669	444.9

<sup>1</sup> Calculated from the equation of the calibration curve (Fig. 2).

TABLE II—*Continued*

Weight of substance 6	Specific gravity of substance 7	$\delta = a/vd$ 8	DK of substance calculated by Lichteneker formula 9	DK of mixture reduced to 100 g. according to our formula 10
135.2	1.439	0.396	12.11	2.270
134.7	1.439	0.395	12.34	2.256
132.7	1.439	0.389	12.40	2.250
133.4	1.439	0.391	12.30	2.250
131.7	1.439	0.386	12.74	2.263

TABLE III

GRANULATED SUGAR, MOISTURE CONTENT 0.55 PERCENT

Readings on "Grobkondensator" 1	Readings on "Feinkondensator" 2	Summary readings in terms of "Grobkondensator" alone 3	DK of mixture of substance and air <sup>1</sup> 4	Weight of condenser with substance 5
49.3	50	49.3	2.138	494.8
49.3	47.0	49.18	2.164	498.4
49.3	47.1	49.18	2.164	497.4
49.3	42.8	49.01	2.201	502.1
49.3	44.8	49.09	2.184	500.8

Weight of substance 6	Specific gravity of substance 7	$\delta = a/vd$ 8	DK of substance calculated by Lichteneker formula 9	DK of mixture reduced to 100 g. according to our formula 10
181.6	0.483	1.588	4.60	1.640
185.2	0.493	1.588	4.79	1.668
184.2	0.490	1.588	4.83	1.671
188.9	0.502	1.588	4.81	1.675
187.6	0.498	1.588	4.82	1.671

<sup>1</sup> Calculated from the equation of the calibration curve (Fig. 2).

Such measurements and calculations were made for every sample tested. Table IV presents the results of the measurements for all the products tested. Each figure is the average of five experiments.

The curves corresponding to these tables are given in Figures 3, 4, and 5. The curves of Figure 3 show the degree to which the DK of the mixture (powder) depends on the moisture content, disregarding compression. The figures under column 2 of Table IV were used to draw the curves. The curves of Figure 4 show graphically the degree to which the DK of the substance calculated by the logarithmic formula depends on moisture content (data under column 3 used). The curves of Figure 5 show the degree to which the DK of the mixture,

TABLE IV  
MEASUREMENTS FOR ALL PRODUCTS TESTED

Moisture percentage (by drying) 1	DK of mixture of the substance and air 2	DK of substance calculated by logarithmic formula 3	DK of mixture reduced to 100 grams according to our formula 4
Biscuit No. 1			
1.89	1.973	5.35	1.775
2.37	1.990	5.38	1.779
9.25	2.561	13.15	2.339
10.76	2.823	16.47	2.529
13.46	3.404	27.27	3.044
15.75	3.719	35.40	3.345
Biscuit No. 2 (finely crumbled)			
3.89	2.198	5.64	1.846
4.66	2.291	6.37	1.921
9.00	2.605	10.45	2.240
10.71	2.937	13.52	2.412
11.59	3.226	15.96	2.592
13.93	3.567	25.60	2.989
Biscuit No. 2 (more roughly crumbled)			
3.18	2.009	6.12	1.839
6.64	2.247	7.63	2.004
9.37	2.562	13.28	2.349
11.61	2.820	18.63	2.603
12.41	3.201	24.34	2.832
13.91	3.438	33.45	3.147
Biscuit No. 3			
3.14	1.918	6.05	1.834
4.79	2.060	6.72	1.919
8.75	2.405	11.28	2.260
14.37	3.512	33.72	3.252
16.77	4.154	64.50	3.950
Flour, 85%			
3.59	2.251	8.40	1.986
4.45	2.300	8.52	1.994
9.60	2.855	13.87	2.350
12.75	3.029	18.74	2.549
13.67	3.050	20.76	2.611
14.67	3.251	23.84	2.735
15.00	3.350	26.38	2.798
Flour, 79%			
3.44	2.133	8.28	1.924
3.84	2.238	8.58	1.981
9.00	2.676	12.38	2.258
12.77	2.921	18.88	2.529
13.84	3.039	21.48	2.624
15.55	3.283	28.18	2.839
16.10	3.322	30.15	2.891

TABLE IV—*Continued*

Moisture percentage (by drying)	DK of mixture of the substance and air	DK of substance calculated by logarithmic formula	DK of mixture reduced to 100 grams according to our formula
1	2	3	4
Flour, 30%			
3.58	2.369	8.78	2.031
5.57	2.502	10.28	2.137
8.10	2.849	12.49	2.299
15.71	3.285	24.64	2.768
17.62	3.370	30.22	2.930
Granulated sugar			
0.05	2.015	3.71	1.553
0.55	2.170	4.77	1.665
1.00	2.307	5.78	1.762

reduced to 100 g. according to our formula (column 4), depends on the moisture content.

Having obtained the experimental results, the next problem was to compare the accuracy with which the moisture content of the powders could be determined by the three methods.

It was essential first of all to know how accurate the determination of moisture content was when no estimate was made of the compression. Since this method is the simplest and most rapid, it would be the most practical, were it to yield good results. If the accuracy of this method be compared with the accuracy of analysis obtained when the formulas were used, appropriate conclusions can be drawn as to the expedience of the recalculation by the formulas. Besides it is essential also to throw light on the problem of the effect which crumbling has in each case.

To determine the accuracy of analysis in cases where the crumbling was identical, we set out in the following way. Each point of the curve showing the degree to which the DK depends on the moisture content represents the average of a number of condenser readings at the indicated moisture content. The difference between the average and the moisture content corresponding to each reading gives the degree to which the result of the given measurement deviates from the average. The maximum difference in the results is the difference between the greatest and the least values. If the arithmetic averages of all the deviations obtained are taken and classified according to kinds of product, then the following data are obtained (Table V).

From these figures it is obvious that when the degree of compression is taken into consideration, the results are considerably more satisfactory. For ordinary pouring the average maximum deviation reaches 0.43% for biscuits, 0.95% for flour, and 0.18% for sugar. But should the data of individual tests be taken instead of average values, these figures are obtained: 1.7% for flour up to 0.9% for biscuits, and up to 0.19% for granulated sugar. This is evidence that

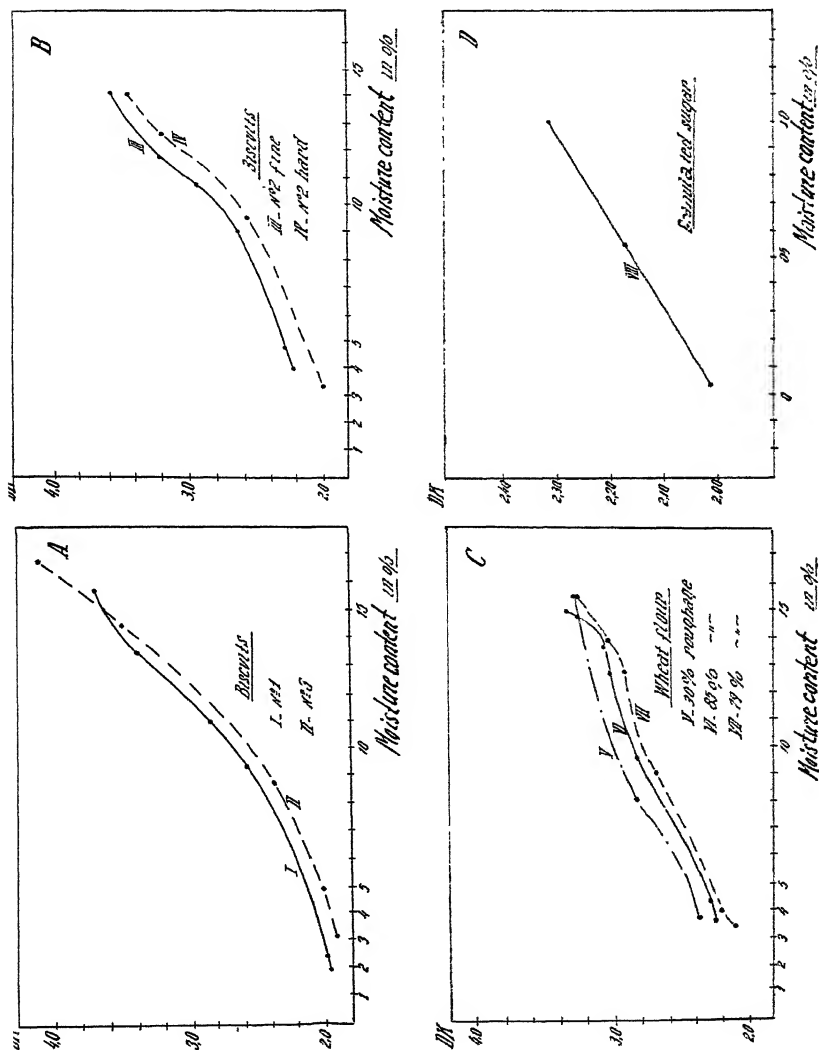


Fig. 2 Relationship of PNE to moisture content

only approximate data can be obtained with such a method. When a recalculation of individual tests is made by the logarithmic formula, the divergence becomes less, reaching 0.40% for biscuits, 0.70% for flour, and 0.06% for sugar. When our formula is applied it becomes still less, reaching 0.20% for biscuits, 0.55% for flour and 0.10% for sugar. Consideration of the degree of compression in the case of crumbled biscuits proves especially effective.

The following conclusion can be drawn from the facts obtained. In cases in which the crumbling is identical the moisture content of

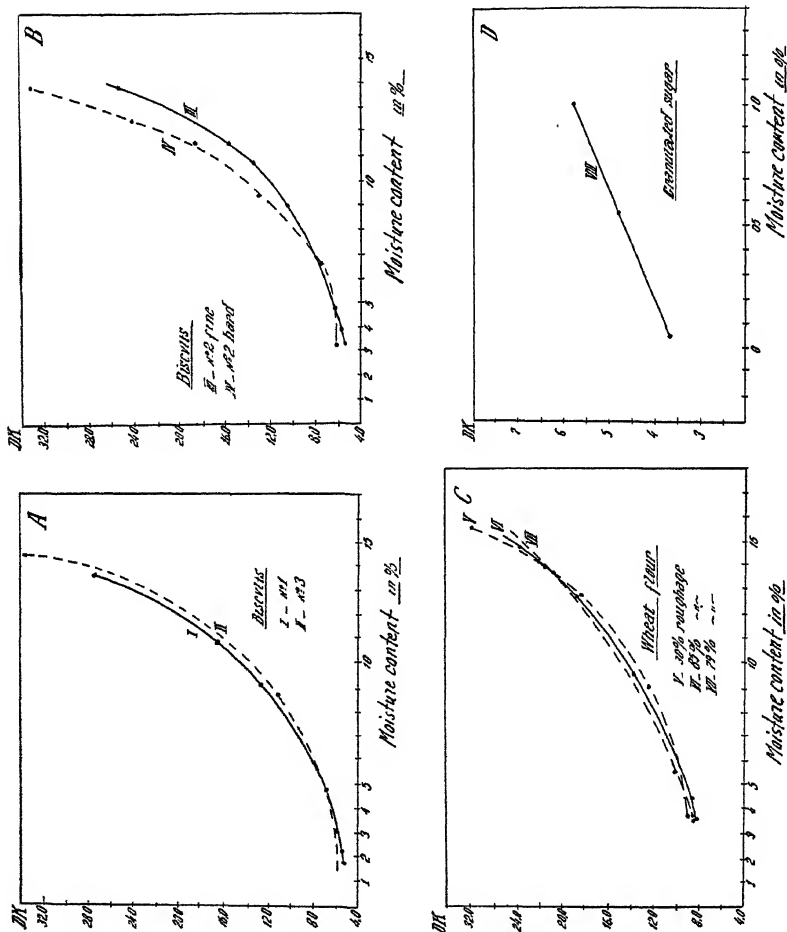


Fig. 4. Relationship of DK calculated by logarithmic formula to moisture content.

biscuits, flour, and granulated sugar can be determined satisfactorily by DK method when calculations are made, either by the logarithmic formula or by our formula. The average deviation from the real (0.09 and 0.07 for biscuits, 0.19 and 0.16 for flour and 0.04 for sugar) is altogether practical for industrial purposes.

A somewhat different picture is obtained when the degree of crumbling varies. From the curves drawn for the two sorts of Biscuit No. 2, which vary in degree of crumbling, one can judge the influence of the size of the powder particles when compression is disregarded (see curve B, Fig. 3). A systematic difference is obtained also when the logarithmic formula is used (see curve B, Fig. 4). Consequently

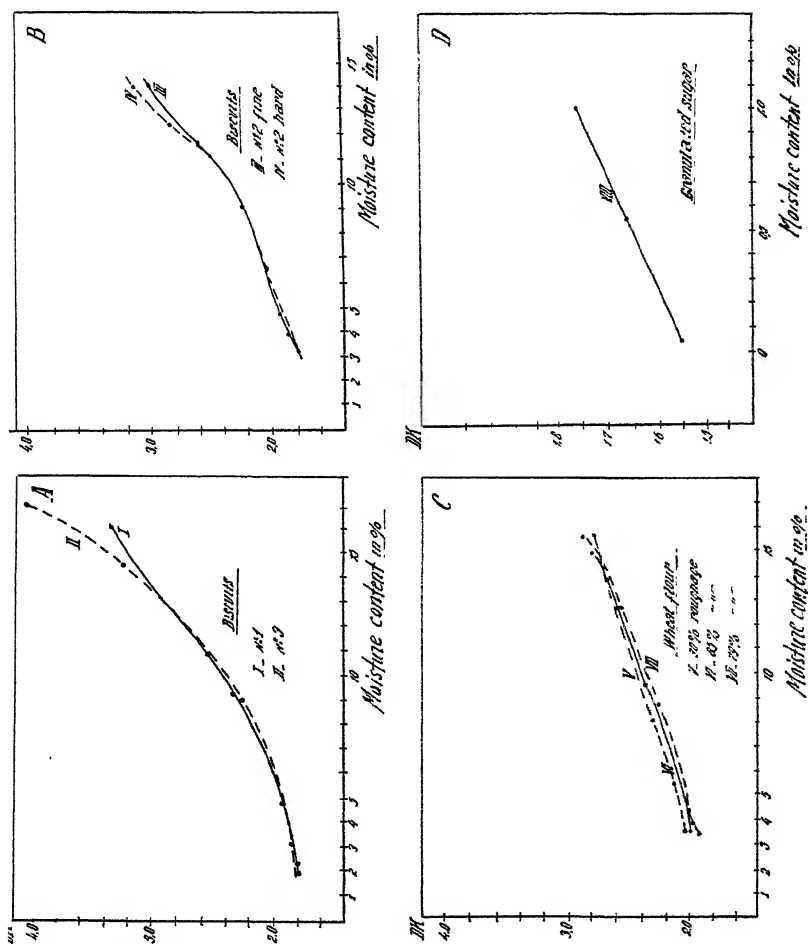


Fig. 5. Relationship of DK reduced to 100 g. to moisture content.



TABLE V  
AVERAGE MAXIMUM DEVIATION IN PERCENTAGE OF MOISTURE CONTENT

Substance 1	Without calculation of compression 2	With calculation by logarithmic formula 3	With calculation by our formula 4
Biscuits	0.43	0.28	0.19
Flour	0.95	0.54	0.48
Granulated sugar	0.18	0.06	0.10

AVERAGE, TAKEN FOR ALL MOISTURE VALUES, OF THE AVERAGE DEVIATION OF  
INDIVIDUAL MOISTURE VALUES IN PERCENTAGE OF MOISTURE CONTENT  
(MEAN AVERAGE DEVIATION)

1	2	3	4
Biscuits	0.20	0.09	0.065
Flour	0.31	0.19	0.16
Granulated sugar	0.05	0.04	0.035

it is apparent that this formula makes no compensation for variations in the degree of crumbling. With moisture contents in the range most probable in practice wholly satisfactory results were obtained with this biscuit only when our formula was applied. This is apparent from the satisfactory coincidence of the curves B of Figure 5.

The problem of determining the moisture content of powders by DK regardless of compression and size of particles must not of course be regarded as fully solved. Considerable work must still be done in testing the applicability of the formulas, in studying the degree to which the results obtained depend on different conditions and factors, on the character and composition of the powder, etc. However, judging from the wholly satisfactory results we obtained, in particular for biscuits, there are grounds to hope for complete success in future work.

It should be noted that apart from our direct purpose, the experimental material can be used also for other conclusions. For instance, the slow rise and subsequent acceleration of DK curves in case of slight moisture content is evidence of the effect of colloids, which combine with water and in this way reduce its DK.

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## REPORT OF THE MALT ANALYSIS STANDARDIZATION COMMITTEE

E. SINGRUEN

Modern Brewer, New York, N. Y.

(Read at the Annual Meeting, May 1938)

The event placing me today into the position of Acting Chairman of the Malt Analysis Standardization Committee of the A. A. C. C. is one that fills us with grief. I refer to the loss of a true friend and always cooperative associate, occasioned by the unexpected and untimely death of Dr. D. A. Coleman. Appointed in 1934 as chairman of the then newly formed Committee on Standardization of Methods for Brewing and Malting Control, Dr. Coleman organized and successfully conducted this important work until his passing last February.

It was my good fortune to have been closely associated with him in this work. Only a few weeks prior to his taking ill, I had the opportunity of visiting him in Washington, at which time he outlined this program and discussed with me the collaborative analytical work to be conducted for the annual report of the committee, which we are about to hear.

In accordance with his plans, five samples of malt with diastatic powers ranging from 40° to 170°L. were sent out to 22 laboratories with the request to determine the diastatic power by the method used for routine tests. The remainder of the samples was to be kept to permit further study of the same malts based on the suggestions offered here today.

Because of the limited time at our disposal in which to conclude this study, it was decided to confine our tests to the determination of the

diastatic power, which had in previous years shown the greatest variations in results.

At this time I take the opportunity of thanking all collaborators who participated in this work for their generous cooperation and for the prompt response necessitated by the rapidly approaching date of this meeting. Of the twenty-two laboratories nineteen sent in their results: among them were seven malt houses, three breweries, three scientific stations, and four government agencies.

After careful study, the results were tabulated, multigraphed, and returned to all collaborators for comment. Thirteen replies were received. Therefore, this report reflects not only the opinion of the committee but includes the comments and suggestions of the majority of the collaborators. The fact is that despite the great strides toward standardization of procedures for the determination of diastatic power in malt, 7 different methods and 4 modifications are in actual use as routine procedures in the various laboratories. This emphasizes the distinct need for agreement on one standard or reference method with which to compare other alternate procedures. The chief variation exists in the determination of the maltose formed.

PARTIAL SUMMARY OF RESULTS FOR DETERMINATIONS OF DIASTATIC POWER  
A.S.B.C. Method for Diastatic Power

Lab. No.	5	6	7	8	17	Max. dif.	Mean
Malt 1	59	45	43	53	51	16	50
2	81	68	69	65	74	16	79
3	111	94	113	110	111	19	108
4	130	129	154	146	136	25	139
5	143	161	179	162	152	36	159

Sallans and Anderson, Ferricyanide Method for Diastatic Power

Lab. No.	3	15	16	Max. dif.	Mean
Malt 1	47	46	46	11	47
2	71	74	73	3	73
3	109	113	112	4	111
4	141	137	137	4	138
5	159	164	158	6	160

By the accumulation of collaborative results obtained by different methods, valuable data have been gathered which permit a better evaluation of the individual procedures. Preference was given to the ferricyanide method as suggested by Sallans and Anderson (Can. J. Research C 15: 70-77, 1937, and Modern Brewer, Nov. 1937) and to the method of the A.S.B.C. In addition gravimetric, tube, and polarimetric methods were employed by individual laboratories on the basis of personal preference or adaptability to existing conditions. If these individual methods are eliminated the mean results of the ferricyanide and A.S.B.C. methods are in close accordance, with less variation in results with the ferricyanide method, both for repeat tests

in one laboratory as well as for comparative tests by different analysts.

Based on the results and comments of the collaborators the committee proposes the following recommendations:

1. That the study of procedures for the determination of diastatic power in malt be continued on the same samples now in the hands of the collaborators before action is taken on adopting one or the other method as standard.

2. That the ferricyanide method as published by Sallans and Anderson be compared with the method of the A.S.B.C. without modifications in either case.

3. That groups be formed according to the preference of different laboratories for a specific method to study the procedures; this would include the polarimetric, gravimetric, and ceric sulfate methods.

4. That all methods, together with their modifications, be made available in either multigraphed or printed form to give all collaborators the benefit of closer study.

## CEREALS USED IN BREWING

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(Read at the Annual Meeting, May 1938)

When we speak of cereals in connection with brewing, we usually think of barley, malt, corn, and rice and give little thought to the fact that practically every known cereal, with the exception of wild rice, at some time or other has been used in the manufacture of fermented beverages comparable with beer.

Cereal beverages have formed an integral part in the diet of the human race throughout the ages. They were the tribal or national beverage of all agricultural peoples and were considered as essential as bread. While bread filled the need for sustaining and energy-providing food, these fermented beverages contributed to the enjoyment of life in the leisure hours. In many cases they contributed greatly to the maintenance of health by taking the place of unsatisfactory or infected water supplies.

They also played an important part in the worship of primitive people and in the celebration of important events and festive occasions such as weddings and births, as well as a religious and national feasts and holidays. The almost universal use of barley malt can probably be attributed to the fact that the culture of barley is possible further

north and at higher altitudes than any other grain—as well as to the comparative ease with which it can be malted.

### Ancient Cereal Beverages

Records of beer brewed with barley date back to 2017 B.C. when the tribes along the Nile at Pelusium made a beverage from it, sweetened with honey. In 1960 B.C., Osiris introduced “zythos,” a fermented beverage made of barley.

During the time of the pharaohs brewing was an important industry in Egypt and many distinctly different brewing formulas have come to us in hieroglyphics, relating in detail materials and brewing processes used. These documentary scripts lead us to believe that red barley was the chief constituent of the “hek,” “hag,” or “bausa” of the old Egyptians. G. Maspers, an Egyptologist, reports that “Beer has always been the favorite beverage of the people. Sweet beer, iron beer, sparkling beer, perfumed and spiced beers were drunk cold or hot, beer of thick, sticky millet like that prepared in Nubia and among the negroes of the Upper Nile.”

The grain was crushed in a mortar and moistened; then lumps of this dough were thoroughly kneaded with the feet in a big pot. The wort resulting from an infusion of this dough was poured through a sieve, consisting of a wide, flat basket resting on a large container. Finally it was poured into high jugs which were sealed with large cones of Nile mud.

“Banzali,” the beer of the natives in modern Egypt, is produced by essentially the same methods as were practiced 3,000 years ago. Barley, wheat, or millet is used in malted or unmalted condition. Color and flavor are imparted by pungent spices. It is unknown whether in old times the spices were added to the beverage as an ingredient or were eaten with the drink.

All Oriental and African races had beverages made from cereals or cereal products, frequently sweetened with honey and flavored with aromatic vegetable substances, such as herbs or spices. In China “kiu” was brewed from “sacrificial millet,” a black variety which is possibly the oldest cereal known to man. The well-known “sake” of the Orient is a rice brew.

### Africa and Asia

The Abyssinians still follow century-old, traditional rites in the preparation of “talla” or “merissa,” as the Arabs call it. Barley is formed into a bread dough, roasted, and ground with raw cereals, boiled and fermented.

Malted “eleusina” is encountered all through the negro countries

as far as the Kaffirs in South and East Africa. This cereal, little known outside of Africa, is cultivated almost exclusively for brewing "bilbil," a perfectly clear, reddish brown, pleasantly bitter drink. Another beer made of eleusina is the "ana" of Equatoria, the Egyptian Sudan. In the French Sudan we find "dolo," brewed from millet, maize, and bananas and fermented with the aid of some roots, resulting in a palatable and spicy beverage.

The Sangonassis of the Congo have their "toko" or "pipi" and a rice beer flavored with hops which is brewed by the missionaries in Kimnerza. In East and Southwest Africa the women do all the brewing; there is "pombe," a millet beer, and also "omalofa" and "metabele," both made from kaffir corn. Today the municipal production of kaffir beer is licensed by the (English) government. Recent papers, published in the *Journal of the South African Chemical Institute*, report the results of a scientific investigation into the methods employed. "Kaffir" beer is produced by a partial fermentation of a millet malt gruel; it contains less than 3% of alcohol, approximately 0.75% of organic acids, and 7%-10% of solids. "Utshvala" is prepared from a mash of malted and unmalted grain, to which ground malted grain is added. Fermentation is allowed to start spontaneously and the mash is then strained. Such beers vary appreciably in composition. They probably are of high dietetic value since considerable quantities of vitamins B and C are present. Another variety, "marevu," has a pleasant acidity but contains no vitamin C.

The ancient Armenians kept their "busa" in large pots underground, sucking it up through long reeds. The Phaeonians in Macedonia brewed "zythos" from barley and "parabia" from millet, while the Arabians made a beer-like beverage from barley bread, raisins, honey, and spices.

When still inhabiting Asia, the Aryans made "sura" from panicum, a species of millet, by adding water, honey, curd, melted butter, and barley. This beverage was called "hura" by the western Aryans, living in Persia.

### Brewing in Europe

During the first century B.C., Diodorus wrote of the Kelts or Gauls inhabiting the Spanish peninsula and the British Isles as follows: "Since the climate is too cold, their country produces neither wine nor olives. For this reason, they prepare for themselves a drink made of barley, the so-called 'cerevisa'—"korina" in Irish.

In the 5th century A.D., Orosius reported on the Hispanians: "They prepare artificially out of the juice of wheat a warming beverage which they call 'celia.' Its fiery vigor is first awakened by the steep-

ing of the grain, which is then dried and worked into flour, whereupon it is added to the milk liquor and fermentation finally imparts a tart taste, as well as the heating and intoxicating quality." In the meantime the inhabitants of Spain had learned to brew beers of good keeping quality; they also used beer yeast for raising bread dough and for cosmetic purposes.

Beer was a favorite beverage in Britain and with all Germanic tribes. Tacitus wrote: "Their drink is a liquor prepared from barley or wheat brought by fermentation to a certain resemblance to wine." They also used oats. In Russia "quass" was made from rye and barley.

The first organized brewing system existed under Charlemagne. From this time dates the first authentic document mentioning brewing as a profession. The first reference to the application of hops is found in the writings of Saint Hildegard, who lived in the 11th century—"If thou desirest to make a beer from oats and hops. . . ." A beer recipe from the Tudor period in England calls for "10 quarters of malt, 2 quarters of wheat, 2 quarters of oats, with 11 pounds of hops."

Oatmeal stouts appear to have been quite popular in England and are still manufactured in Australia. The Bohemian beers were originally brewed from barley malt. Wheat or "weiss" beer was not heard of until the 15th century, and then the privilege of making it was reserved to the nobility and those who brewed for the authorities.

### Native American Beers

According to a recent article in the *American Anthropologist*, entitled "Native American Beers," "there is ample evidence of a wide distribution of undistilled, alcoholic beverages, both beers and wines, in North as well as South America." Contrary to the old world, where barley, oats, millet, and similar grains were the most commonly used brewing materials, corn was the predominant cereal crop in the western hemisphere, from where it was introduced into other parts of the world. In both Americas numerous distinctly different strains of corn were grown, among them white, yellow, red, and blue varieties.

A most peculiar and to us little appealing procedure was used by the South American Indians to start fermentation, according to Karsten, author of *Civilization*. Instead of employing yeast or any other ferment, they chewed the corn or a baked cornmeal mush, believing that "the saliva, which shares the natural magic power of the whole body, was supposed to favorably influence the spirit that is active in the fermented drink."

The "chicha" of the South American Indians was made from boiled maize, chewed and fermented in large pots, covered with leaves, or in

cowhides spread over poles. This type of beer was known over wide territories, including Mexico, Guatemala, Ecuador, Peru, Chile, and the Andes. The drink of the Nicaraguan tribes was "mazamorro," made of a mixture of honey and ground corn. The natives of Juma roasted wheat grains over a charcoal fire until light brown in color, pulverized them, made a thick mash with water which was fermented. Of all grain-growing tribes only the Pueblos lacked fermented beverages, whereas many of their nomad neighbors had them.

### Early American Brewing

Equally fascinating is a study into the historical background of our own brewing materials. Upon their arrival in the new world, the first settlers were faced with a lack of proper grains for malting. Therefore every newcomer was requested by decree to bring with him a certain amount of malt from England. Lack of grain, however, did not discourage people from brewing their favorite beer or ale, as this old colonial rhyme shows:

"If barley be wanting, to make into malt,  
We must be contented and drink it no fault,  
For we can make beer to sweeten our lips,  
From pumpkins, and parsnips and walnut-tree chips."

For this reason, the suitability of Indian corn as a brewing material became one of the first research problems of cereal chemistry in this country. In his book, *The Advancing Front of Science*, G. W. Gray writes:

"It was in 1635 that the science obtained its first foothold in the New World. In that year John Winthrop, Jr., a young alchemist of the Massachusetts Bay Colony, visited England and obtained from the Crown a commission to develop certain native mineral resources. He was interested in the production of copper, glass, iron, lead, tar, and other 'chymicals,' including medicines. The Royal Society asked him to see if the grain, American maize, would produce beer. Winthrop tried it and brewed a 'pale, well-tasted middle beer.' He even did research on cornstalks and found that they yielded syrup sweet as sugar, a foretaste of the extensive corn syrup industry of today."

Ever since 1641 attempts were repeated to make malt from Indian corn, but never with any success. However, corn was used in other forms, and in 1662 Winthrop delivered a lecture before the Royal Society of London on the question of brewing with corn and in 1663 he treated the dignitaries with the first beer brewed with corn in Europe. It is reported that it met with great favor.



Both wheat and oats were grown by the early Dutch settlers in New York State for brewing purposes, while much malt was imported from European countries, particularly England, because no proper malting facilities were in existence here. The poorer classes, not being able to afford the costly imported barley malt, brewed their beer with molasses and bran. In New England sassafrass, boiled with roots or herbs, birch, spruce, or sassafrass bark, with pumpkins and apple parings, sweetened with molasses, maple syrup or beet tops substituted for malted grain in times of need. Poor grain crops occasionally were responsible for temporary prohibition periods; for instance, in 1641 Massachusetts prohibited the use of wheat for bread and malt in order to promote export.

In 1641 John Appleton of Massachusetts built what was probably the first malt house, as well as a brewery in Watertown, where he experimented with Indian corn. New England malt gained reputation and soon was "exported" to the other colonies, particularly Pennsylvania. The first patents of the manufacture of corn beers, brewed with unmalted corn, were granted to Alexander Anderson of Pennsylvania on January 26, 1801.

Malt adjuncts in various forms came on the market about 1850. Secret brewing procedures with raw grain were offered to brewers at exorbitant prices. The improperly prepared materials (patents to Fred Seitz, 1852-70) made from the whole kernel became rancid rapidly, imparting a bad flavor to the mash which had to be removed with bone black. This situation was improved by replacing yellow corn with white varieties and by removing the germ and husk. Satisfactory results, however, were not obtained until the whole problem was investigated scientifically, after which rapid progress was made.

"Cerealine," the first corn flake manufactured from shelled, deoiled, and ground corn by the application of heat and moisture, made its appearance on the market in 1883. "Frumentum," produced in strictly mechanical manner by the dry process, followed in 1891.

During the second half of the 19th century, the agricultural importance of brewing materials grew with the rapidly expanding brewing industry, and the cultivation of barley spread with the settlement of the Middle West, where it was grown with increasing success after 1860 in Ohio, Wisconsin, Iowa, and after 1870 in Minnesota, Nebraska, Utah, and later in the Dakotas and California. From 1861 on the Manchurian varieties were introduced in the Middle West.

However, it was not until the beginning of the 20th century that a barley-improvement program was organized by the Department of Agriculture in cooperation with the malting and brewing interests. At that time, there existed a controversy about the brewing value of

imported two-rowed barley malt as compared with American six-rowed varieties, leading to an extensive study of American barleys and malts, the results of which were published by the Department of Agriculture as Farmers' Bulletin No. 124, entitled "Chemical Studies of American Barleys and Malts," by J. A. LeClerc and R. Wahl.

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## BARLEY CORRELATION VALUES FROM SIX STATES, COMPARING CHEMICAL VALUES

JOSEPH C. IRELAND and HUGO O. GRAUMANN

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(Received for publication December 16, 1938)

Barley has not been considered a commercial crop in Oklahoma, and many farmers and experiment station investigators seem to doubt the advisability of trying to increase the crop. Seeded in September or October, a very desirable winter pasture is provided, and the grain is harvested in May or June, before the heat of summer injures the quality. One or two species of small barleys are indigenous to Oklahoma, and the acreage of the cultivated crop is increasing each year. The object of this study has been to determine the relative commercial value of Oklahoma barley.

Specialists from California, Colorado, Michigan, Minnesota, and Wisconsin have very generously supplied us with samples of their most popular varieties to make a study of the comparative quality of Oklahoma selections with their barleys.

The weight of 1,000 kernels, the diastase, catalase, total nitrogen, and the hydrolyzable solids were considered most expressive of the commercial qualities of barley, as shown by Shellenberger and Bailey (1936). Catalase determinations have not been used extensively in determining the germinative power of barley, but Davis (1926) and Legatt (1929) indicate it to be a very effective method of estimating the germinating capacity of other seeds. Since the germination percentage has certain physiological limitations, catalase measurements have been substituted as a quantitative method of expressing the germinating power of the kernels.

The comparison of the results of the various estimations has been based upon multiple correlation values. Tabulated results eliminate the speculative relationships and make possible the reproduction of a similar study by other investigators.

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The comparison of the results of the various estimations has been based upon multiple correlation values. Tabulated results eliminate the speculative relationships and make possible the reproduction of a similar study by other investigators.

### Methods

The barley samples are listed in Table II, which includes the names of the varieties and the states from which they were obtained. Several of these have been used by other workers and are well known. One thousand kernels were counted out for each sample and weighed in grams. Samples of 100 grams were cleaned and ground with the finest plates of a small food chopper. This material was used for the following determinations.

Five-gram samples of the ground grain were used for diastase determinations, 2-gram samples for total nitrogen, 1-gram samples for catalase, and  $2\frac{1}{2}$  grams for hydrolysis. These were treated as directed in the Official Methods of the A. O. A. C. For catalase, the methods of Davis (1926) were used, with the exception of grinding.

The methods of Wallace and Snedecor (1931) were used in making the multiple correlation study. Tabulations were made after all estimations had been carefully repeated.

### Results

Differences among barleys from the various experiment stations are shown in Table I. Kernel weights in Table I indicate that Cali-

TABLE I  
AVERAGE VALUES OF BARLEY VARIETIES

State	Hydrolyzed solids	Total nitrogen	Catalase	Diastase	Wt. 1,000 kernels
	%	%	cc. O <sub>2</sub>	mg. maltose per 10 g.	g.
Oklahoma	70.50	2.16	32.45	148.00	30.11
Wisconsin	70.40	1.78	27.76	112.00	26.60
California	67.40	1.74	21.72	72.80	41.48
Minnesota	67.20	1.96	32.12	126.80	29.14
Colorado	70.20	2.20	28.72	121.60	32.48
Michigan	71.40	1.64	29.70	144.00	36.40

fornia barleys are quite superior in plumpness and development of grains, but the other determinations for the California samples averaged very low. This is especially true with the enzymes. On the other hand, Michigan barleys have heavy, well-developed kernels, a high percentage of carbohydrate, low nitrogen, and high diastase. Oklahoma barleys are very poor in appearance, compared with those from the two states mentioned above, but the diastase and catalase content are unusually high. The first part of Table II shows the details for each variety.

Some interesting observations may be made of the results shown in

Table II. The highest percentage of hydrolyzable solids may be found in Michigan Winter barley, grown in Oklahoma. It also has a comparatively low total nitrogen content. It is to be noted that this barley is the highest yielding and one of the most promising types grown in Oklahoma and that it is not grown at the Michigan Experiment Station. Very near to it in yield and popularity is Missouri Beardless, high in diastase and hydrolyzable solids, but lacking in grain plumpness.

TABLE II  
MULTIPLE CORRELATION TABLE OF FOUR INDEPENDENT VARIABLES, USING THE WEIGHTS OF 1,000 KERNELS AS THE DEPENDENT VARIABLE

Variety	Hydrolyzed solids	Total nitrogen	Catalase	Diastase	Wt. 1,000 kernels
	%	%	cc. O <sub>2</sub>	mg. maltose per 10 g.	g.
<i>Oklahoma</i>					
1. Mich. Winter	77	1.8	33.9	94	35.4
2. Composite	73	2.0	27.3	150	34.5
3. Hero	68	2.2	36.0	68	33.3
4. Heron	69	2.0	33.9	126	32.0
5. Comfort	70	2.4	33.4	232	30.8
6. Black Smyrna	66	2.3	28.6	162	30.4
7. Missouri Beardless	71	2.2	31.0	204	23.2
8. Trebi	70	2.4	35.5	148	21.3
<i>Wisconsin</i>					
9. Trebi	75	1.7	26.2	120	34.6
10. Peatland	71	2.0	27.5	94	27.6
11. Wisconsin Beardless	69	1.7	30.0	124	25.0
12. Velvet	65	1.8	27.0	120	23.6
13. Oderbrucker	72	1.7	28.1	102	22.2
<i>California</i>					
14. Vaughn	65	1.6	22.2	68	48.3
15. Club Mariout	68	1.7	20.2	78	43.9
16. Tennessee Winter	69	1.6	19.1	66	42.2
17. California Coast	65	2.1	23.3	72	36.9
18. Hannchen	70	1.7	23.8	80	36.1
<i>Minnesota</i>					
19. Trebi	66	2.0	29.5	136	31.7
20. Wisc. No. 38	68	1.9	36.0	102	30.3
21. Velvet	71	1.9	29.4	110	28.9
22. Glabron	64	2.0	33.0	152	27.6
23. Manchuria	67	2.0	32.7	134	27.2
<i>Colorado</i>					
24. Club Mariout	72	2.2	26.9	110	26.6
25. Flynn	73	2.2	30.3	124	27.1
26. Velvet	68	2.4	31.6	122	34.6
27. Trebi	68	1.8	24.1	152	42.5
28. Colless	70	2.4	30.7	100	31.6
<i>Michigan</i>					
29. Alpha	73	1.7	28.9	140	40.6
30. Trebi	74	1.4	26.6	150	40.1
31. Michigan Two-Row	72	1.8	38.8	160	36.4
32. Velvet	68	1.8	26.1	144	32.5
33. Wisc. No. 38	70	1.5	28.1	126	32.4
Sum	2297	63.9	959.7	4070	1071.4
Mean	69.6	1.94	29.1	123.3	32.5

TABLE II—*Continued*  
SOLUTION OF NORMAL EQUATIONS

Variable	Hydrolyzed solids A	Total nitrogen B	Catalase C	Diastase D	Wt. 1,000 kernels X
		%	cc. O <sub>2</sub>	mg. maltose per 10 g.	g.
A	—	-.1154	+.0516	+.1253	-.1363
B	—	—	+.3164	+.2235	-.3615
C	—	—	—	+.4169	-.5523
D	—	—	—	—	-.1364

By the normal-equation method R was found to be +.308, which is probably not significant.

The general appearance and weight of 1,000 kernel would indicate that the California barleys are quite superior to any other. The low total nitrogen would indicate that the proteins are not offensive in malt production. The low diastase and catalase content of these barleys is also noteworthy. The entire group of Michigan barleys have a correspondingly low total nitrogen content, a high diastase content, and very plump grains.

Trebi seems to be one of the most extensively grown experiment-station barleys. The hydrolyzable material is comparatively high in each group. In the Michigan group, Trebi has the lowest total nitrogen content of any selection and is high in hydrolyzable content, as well as in diastase. The fact that this variety is grown in five of the six states and that it has all the qualifications of a superior malting barley would indicate that Trebi is one of the best commercial barleys.

Dickson and others (1938) have the following statement to make regarding Trebi: "Trebi (C. I. No. 936) is a six-rowed, rough-awned variety and has large blue kernels. It is a selection from an imported lot of seed obtained by the United States Department of Agriculture from the south side of the Black Sea in 1905. The selection was made in 1909 by H. V. Harlan, of the United States Department of Agriculture, at the Minneosta Agricultural Experiment station. It was released for commercial production in 1918."

In the second part of Table II, in the solution of normal equations, line A, it may be observed that the correlation coefficients of nitrogen, catalase, diastase, and kernel weight are not especially significant, when compared with hydrolyzable solids. In line B, the comparison is made with the total nitrogen. It may be observed that the catalase and diastase have some significance and that the kernel weight is negative. The same is true in line C, in which we have a positive correlation of catalase and diastase, with a negative value for kernel weight. The multiple correlation, +.308, is probably not significant.

### Summary

Multiple correlation data of the hydrolyzed solids, total nitrogen, catalase, diastase, and weight of 1,000 kernels are presented for barleys from six states. The coefficient of multiple correlation is of doubtful significance.

The correlations of 1,000-kernel weights with total nitrogen and diastase estimations are generally negative.

A general conclusion of the tabulation is that the higher the weight of grain is, the greater is the error of estimate.

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## THE WHEAT-MEAL-TIME FERMENTATION TEST. III. EFFECT OF BRAN, PROTEASES AND ACTIVATORS ON THE "TIME" OF FLOUR<sup>1</sup>

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(Received for publication November 10, 1938)

In a previous paper (Swanson, 1937) it was shown that the addition of bran to flour made the "time" different from that obtained on meal. Why the mixing of bran or shorts with the flour in the same proportion as these substances are found in the meal should make the time longer is a problem for study. Since it is the flour which is used in baking, it would seem more logical that the "time" test should be made on flour rather than the meal. There are also indications that the "time" is influenced by substances present in the parts of the wheat kernel which are removed in the milling process. This situation raises a further ques-

<sup>1</sup> Contribution No. 58, Department of Milling Industry.

<sup>2</sup> Credit is due Glen West, student assistant, for able and painstaking work in performing the details of this time test.



tion as to the value of this test for indicating quality in flour. Since the effects of bran added to flour are very different from the effects of bran in the meal, it was thought worthwhile to make these tests on a wider scale than was done previously. Swanson and Dines (1939) showed that the proteases shorten the "time" and that this effect is much greater on long "time" than on short "time" wheats. Since bran and proteases have opposite effects, these substances were used in combination and protease activators were also tried in the present investigation.

The findings in this and previous papers are of significance because of the possible usefulness of this test to plant breeders, since "time" is apparently a variety characteristic. However, if the "time" can be lengthened or shortened by the addition of certain substances, especially those from the wheat itself, the question arises whether this is really a test of gluten quality or a test of the presence or absence of the substances which influence the "time." It is evident that before the figures obtained in this test can really be evaluated in respect to wheat quality, it is necessary to know the factors which determine the number of minutes which will elapse between the moment the doughball is put in the water and the breaking on the under side is observed.

### Wheats Used

The two hard red winter wheats, and flours from the same, used in much of this investigation were Tenmarq, representing long "time," and Chiefkan, representing short or medium "time," of the crop of 1937. A 2,000-gram sample of each was milled, so as to get flour, bran, and shorts for these trials. The wheat meal was ground in a hammer mill to pass a  $\frac{1}{2}$ -mm. sieve. The bran was also ground in the same manner. Additions to either meal or flour were mixed in beforehand so as to be thoroughly incorporated in the dough while this was mixed. The further details of performing the test have been given in the papers just cited.

The data obtained on the meals and flours from these two wheats are given in Table I. These figures were the averages of several trials on the meals and flours and may therefore be used for a general reference. It will be observed that the figures for the checks in the several tables given in this paper are not always the same as those in Table I. The variations are due to experimental errors and their magnitudes must be considered in making comparisons.

It is very evident that the "time" on the flours is much longer than on the meals and that the differences between the flours from these two varieties are less than between the meals. However, on the Tenmarq

TABLE I  
TIME ON MEAL AND FLOUR FROM TENMARQ AND CHIEFKAN

Material	Tenmarq	Chiefkan
	<i>Min.</i>	<i>Min.</i>
Meal	121	59
Flour	132	111

flour the "time" is longer than on the Chiefkan flour. Hence these two wheats and their flours were suitable for these experiments.

It is much more difficult to obtain uniform readings on doughballs made from flours than on those made from meals, because the end point is more obscure at the flour doughball-water interface. For this reason the experimental error on flour is greater than on meal.

### Effect of Adding Bran to Flour

In each case the bran from Tenmarq was mixed with Tenmarq flour and bran from Chiefkan mixed with the Chiefkan flour. Since the bran was ground on the same hammer mill as was used for grinding the wheat meal, the bran was of the same fineness but freer from endosperm than the bran flakes in the meal. The results obtained are given in Table II.

TABLE II  
INCREASE IN "TIME" DUE TO ADDING BRAN TO FLOUR

Proportions in mixture	Tenmarq		Chiefkan	
	Total	Increase	Total	Increase
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
15 g. flour (check)	137	—	110	—
14 g. flour+1 g. bran	182	45	148	38
13 g. flour+2 g. bran	194	57	174	64
12 g. flour+3 g. bran	205	68	168	58
11 g. flour+4 g. bran	—	—	171	61

It is evident that the effect of the bran when separated in the ordinary milling process is different from that of the branny material in the meal. This is not due to the granulation of the bran flakes, because the bran was ground in the same hammer mill as the meal. The longer "time" on flour in comparison with the meal might be explained on the basis of a denser gluten structure in the flour doughball. Mixing in the bran would dilute this gluten structure and hence the "time" should be shortened. Instead it is longer, and is still longer with the larger amounts of bran which would dilute the gluten structure all the

more. It seems therefore that the lengthening is due to some substance in the bran which inhibits the factors which cause the disintegration of the doughball. However, this inhibition is not effective when the bran material is in the meal. The meal differs in two important respects from the mixture of bran and flour. In the meal the particles of endosperm are coarser and the germ is also present. That the granulation alone does not account for all this difference was shown in the following trials.

### Effects of Granulation

Granulation of the meal may be affected by tempering so as to have a softer endosperm and also by regrinding the meal first ground in the hammer mill. The wheat was tempered at 14%, 16%, and 18% moisture. The regrinding was done by passing the meal first ground in the hammer mill, through the corrugated rolls of the Allis mill set as for the fourth or last break. The material was then sifted over 30 grits gauze and the throughs ground on the smooth rolls set as for the third middlings reduction. This was then sifted over the 10XX and the overs were again ground between the smooth rolls set as for the last reduction. This would reduce the endosperm to almost the same fineness as in flour. The results from these various grindings are given in Table III.

TABLE III  
EFFECT OF GRANULATION OF THE MEAL ON TIME

Treatment	Tenmarq	Chiefkan
	<i>Min.</i>	<i>Min.</i>
Ground once in hammer mill	121	59
Meal reground in Allis mill	137	82
Tempered at 14%, ground once in hammer mill	129	64
Tempered at 16%, ground once in hammer mill	117	65
Tempered at 16%, meal reground in Allis mill	139	92
Tempered at 18%, ground once in hammer mill	118	61
13 g. flour+2 g. ground bran	194	174

While the "time" is increased by the finer granulation, it does not become as long as when bran is mixed with the flour.

### Effects of Shorts and Germ Stock

Shorts contain bran material in a finely pulverized condition and also considerable endosperm from near the bran coat. The germ stock was a mill stream containing about 10% germ material mixed with the shorts in the usual milling process. The shorts were mixed with the

flour in a manner similar to that of bran so that the shorts from Tenmarq wheat were mixed with the Tenmarq flour and the same procedure was used with Chiefkan. The results obtained are given in Table IV.

TABLE IV  
INCREASE IN "TIME" DUE TO SHORTS AND INCREASE OR  
DECREASE DUE TO GERM STOCK

Material	Tenmarq		Chiefkan	
	Total	Increase or decrease	Total	Increase or decrease
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
15 g. flour (check)	137	—	110	—
14 g. flour+1 g. shorts	183	46	136	26
13 g. flour+2 g. shorts	221	84	180	70
12 g. flour+3 g. shorts	257	120	183	73
14 g. flour+1 g. germ stock	180	43	145	35
13 g. flour+2 g. germ stock	160	23	70	-40
12 g. flour+3 g. germ stock	79	-58	54	-56
10.5 g. flour+2.3 g. bran+2.2 g. shorts	275	138	220	110

A comparison of the figures in Tables IV and II shows that shorts lengthen the "time" on Tenmarq as much as bran, or even more. Shorts contain endosperm material from near the bran coat similar to fourth-break flour. This flour has a longer time than the other mill-stream flours (Table VI). This together with the possible inhibiting effect of bran would account for the longer time obtained with the mixture of Tenmarq shorts and flour. The increase in time from one gram of germ stock which would contain more germ than present in 15 grams of meal from the whole wheat is probably due to the inhibiting effect of the branny material and to endosperm material similar to that in the shorts. The shortening of the "time" by larger amounts of germ stock may be due to a protease in the germ or to the phosphatides present in the germ, or possibly both.

### Influence of the Germ

That the germ does have a shortening effect on "time" was further shown by making the determination on meal from which the germ had been removed, in comparison with meal containing double the amount of germ naturally present and meal ground from the whole kernel. The Tenmarq and Chiefkan used in this trial were not from the same lots as those used in the other trials reported in this paper, but the time of the former had been found to be twice that of the latter. A small lot of each was used for dissection and the germ end of each

kernel was cut off with a sharp scalpel while the kernel was held by tweezers under a four-inch magnifying glass. After this separation the portions were weighed and ground in a coffee mill. Whole-wheat portions were also ground in the same mill so as to minimize differences due to method of grinding. The data obtained in this trial are found in Table V.

TABLE V  
EFFECT OF GERM ON THE "TIME"

Material	Tenmarq		Chiefkan	
	Percent of kernel	Time	Percent of kernel	Time
		<i>Min.</i>		<i>Min.</i>
Whole meal	100	82	100	41
Brush ends	83	118	81	62
Germ ends	17	—	19	—
Whole meal+germ	—	51	—	32

The amounts obtained from the germ ends were too small for a separate determination. The proportions in whole meal + germ were the same as obtained in the dissection. It is very evident from the data that the "time" on meal from which the germ has been removed is considerably longer than on meal from the whole kernels. It is also evident that when germ is added so that the meal contains twice as much germ as originally present, the time is considerably shorter. That is, when the germ is removed the "time" is longer and when added it is shorter. Thus the germ contains some substance, probably a protease or a phosphatide, which greatly influences the "time."

That there is a variation in "time" on material obtained from different parts of the kernels was shown by making the determination on various mill stocks. The samples described in Table VI were obtained from milling locally grown Tenmarq wheat. All the products except the flours were reground in the hammer mill so as to have the usual degree of fineness. It is very evident that too much branny material such as is present in second- and third-break stock weakens the gluten structure and hence shortens the "time." The various flours as well as the middlings, except the first tailings, all have longer "time" than the wheat meal when bran is added to the flour; the time, however, is longer than on the meal. Regrinding the middlings would reduce them to nearly the same fineness as the flour. The shorter "time" on the first tailings is probably due to the presence of the germ, most of which goes into this stream. The sizings also contain considerable germ, which probably causes the shorter time. When the germ material is

TABLE VI  
TIME ON WHEAT AND VARIOUS MILL STOCKS

Material	Time
	<i>Min.</i>
Wheat above first-break rolls	89
Stock above second-break rolls	67
Stock above third-break rolls	33
Sizings, above rolls	82
First middlings above rolls	100
Third middlings above rolls	124
Fifth middlings above rolls	116
First tailings above rolls	84
First-break flour	105
Fourth-break flour	139
Sizings flour	117
Third-middlings flour	102
First-tailings flour	121
Straight flour	124
12 g. straight flour + 3 g. bran	132

removed by sifting, as is the case with the sizings and first-tailings flour, the time corresponds in length to that of other flours.

That the germ has a tendency to shorten the time thus appears from three trials: (1) the addition of germ stock to flour, (2) the increase in the germ content of the wheat meal, (3) the shorter time obtained on mill streams which contain the germ as compared with those which do not.

#### Effect of Water-Extracted Bran

It seems that the lengthening of the time by bran and shorts when mixed with flour is due to some inhibiting substance. To see if this can be removed by water extraction, 35 g. of bran of each variety was stirred up in 420 c.c. of water and allowed to soak over night. This was then filtered on linen cloth, washed with several portions of water, and drained between washings. The bran was then spread out in a thin layer until air dry, and then ground in the hammer mill. The results obtained by mixing portions of this extracted bran with flour in making the doughballs are given in Table VII.

The results obtained indicate that all of the active substance was removed by the water extraction from the Chiefkan bran and only partly from the Tenmarq bran. That is, after water extraction the Chiefkan bran acts merely as so much inert material. In the previous investigation (Swanson, 1937) it was shown that inert substances such as paper pulp and alundum powder had no effect on the "time" of flour. Whether a more thorough extraction of the Tenmarq bran might have made this inert also was not determined, but it is evident that the bran

TABLE VII  
EFFECTS OF WATER-EXTRACTED BRAN

Material	Tenmarq		Chiefkan	
	Total	Increase	Total	Increase or decrease
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Flour alone, check	132	—	111	—
14 g. flour+1 g. extracted bran	157	25	110	-1
13 g. flour+2 g. extracted bran	174	42	112	1
12 g. flour+3 g. extracted bran	164	32	103	-8

from the two wheats behaved differently after the process of water extraction.

### Effect of Water Extract of Bran

To determine what effect the water extract of bran has on "time," 50 g. of bran of each variety was placed in 200 c.c. of water and allowed to soak over night. This proportion was used because it requires about four grams of water to wet thoroughly one gram of bran and leave enough liquid that can be used for the tests. Four c.c. of the extract thus represented one gram of bran. The soaked bran was placed on linen cloth and as much of the extract as possible squeezed out. This extract was used in amounts and with the results given in Table VIII.

TABLE VIII  
DECREASE IN "TIME" DUE TO WATER EXTRACT OF BRAN

Material	Tenmarq		Chiefkan	
	Total	Decrease	Total	Decrease
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Flour alone	132	—	111	—
15 g. flour+2 c.c. extract ( $\frac{1}{2}$ g. bran)	93	39	88	23
15 g. flour+4 c.c. extract (1 g. bran)	116	16	88	23
15 g. flour+6 c.c. extract ( $1\frac{1}{2}$ g. bran)	124	8	93	18
15 g. flour+8 c.c. extract (2 g. bran)	116	16	94	17

Since the extract of bran decreases the time of both varieties, there seems to be an activating substance which is present in the water extract of bran. It should be noted, however, that the progressively larger amounts of extract do not have a proportionally greater effect. Thus the extract of bran added to flour in making the doughball has effects opposite to that of untreated bran. The soaking over night apparently stimulates some activator, or phosphatides may be hydrolyzed. In a

previous investigation (Swanson, 1937) an increase in time was obtained from the use of the extract of bran but the method of making the extract was not the same in the former trial.

### Effect of Pepsin on Mixtures of Flour and Bran Materials

That pepsin has a distinct effect in shortening "time" has been shown by Swanson and Dines (1939). It was desired to learn whether pepsin would overcome the retarding effect of the bran. Hence trials with bran, extracted bran, and bran extract were made using 2 mg. of pepsin for each 15 g. of mixture. The results obtained are given in Table IX. The figures obtained without pepsin are repeated from the preceding tables for comparison.

TABLE IX  
EFFECT OF PEPSIN ON MIXTURES OF FLOUR AND BRAN MATERIAL

Material	Tenmarq			Chefkan		
	Without pepsin	With pepsin	De-crease	Without pepsin	With pepsin	De-crease
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
Wheat meal	121	27	94	59	32	27
Flour	132	67	65	111	62	49
<i>Untreated bran</i>						
14 g. flour+1 g. bran	182	58	124	148	51	97
13 g. flour+2 g. bran	194	80 <sup>1</sup>	114	174	49	125
12 g. flour+3 g. bran	205	38	167	168	39	129
<i>Extracted bran</i>						
14 g. flour+1 g. ext. bran	157	72	85	110	48	62
13 g. flour+2 g. ext. bran	174	43	141	112	44	68
12 g. flour+3 g. ext. bran	164	30	134	102	37	65
<i>Bran extract</i>						
15 g. flour+2 c.c. ext.	93	52	41	88	78	10
15 g. flour+4 c.c. ext.	116	59	56	88	80	8
15 g. flour+6 c.c. ext.	124	62	62	93	64	29
15 g. flour+8 c.c. ext.	116	68	48	94	70	24
15 g. flour+2 c.c. ext. <sup>2</sup>	93	62	31	88	79	9
15 g. flour+4 c.c. ext.	116	66	50	88	78	10
15 g. flour+6 c.c. ext.	124	69	55	93	77	16
15 g. flour+8 c.c. ext.	116	76	40	94	77	17

<sup>1</sup> Since this figure is so far out of line, an experimental error is probable.

<sup>2</sup> Four mg. pepsin was used in the last four trials.

When pepsin is added together with the bran the time is much decreased as compared with flour alone and the decrease is greater with the larger amounts of bran. This is in contrast with the use of bran alone, which when mixed with flour increased the time and the increase



was greater with the larger amounts of bran. While bran alone retards the time for the breaking of the doughball, the bran plus pepsin shortens the time more than does pepsin alone. Also when both bran and pepsin are added the time on both Tenmarq and Chiefkan are practically the same. The pepsin, together with the extracted bran, shortens the time very markedly and with the largest amount of bran there is no essential difference between Tenmarq and Chiefkan flours. The effect of the bran extract when used with pepsin is less than that of the extracted bran, but here also the differences in time on Chiefkan and Tenmarq flours are negligible. Using 2 or 4 mg. of pepsin seems to make very little difference on the results.

### Effect of the Protease Activator Cysteine-Monohydrochloride

In the previous paper (Swanson and Dines, 1939) it was shown that the protease activator, anhydrous cysteine-monohydrochloride, decreased the time on the meal from a long "time" wheat but not on a short "time" wheat. The trials with the activator were repeated and it was used in 2 and 4 mg. amounts for each 15 g. of flour or mixture. The results obtained with various combinations are given in Table X.

TABLE X  
EFFECT OF THE PROTEASE ACTIVATOR CYSTEINE-MONOHYDROCHLORIDE

Material	Tenmarq			Chiefkan		
	Without C-M HCl	With C-M HCl	De- crease	Without C-M HCl	With C-M HCl	De- crease
	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>	<i>Min.</i>
15 g. meal	121	89	32	59	39	20
15 g. flour	132	113	19	111	100	11
14 g. flour+1 g. bran	182	141	41	148	75	73
13 g. flour+2 g. bran	194	181	13	174	121	53
12 g. flour+3 g. bran	205	175	30	168	121	47
<i>Extracted bran</i>						
14 g. flour+1 g. ext. bran	157	117	40	110	88	22
13 g. flour+2 g. ext. bran	174	94	80	112	86	26
12 g. flour+3 g. ext. bran	164	97	67	103	77	26
<i>Bran extract</i>						
15 g. flour+2 c.c. extract	93	80	13	88	84	4
15 g. flour+4 c.c. extract	116	85	31	88	93	+5
15 g. flour+6 c.c. extract	124	91	33	93	101	+8
15 g. flour+8 c.c. extract	116	101	15	94	123	+29
15 g. flour+2 c.c. extract <sup>1</sup>	93	82	11	88	88	0
15 g. flour+4 c.c. extract	116	90	26	88	96	+8
15 g. flour+6 c.c. extract	124	93	31	93	107	+14
15 g. flour+8 c.c. extract	116	103	13	94	109	+14

<sup>1</sup> Four mg. C-M HCl added in the last four trials.

The cysteine-monohydrochloride reduced the time in all cases except with the bran extract of Chiefkan, which produced an increase, but the observed reduction in time was much less than with pepsin. The reduction in time for flour alone was less than for either the meal or the flour plus bran, which indicates that flour contains very little of the substance which may be stimulated by the protease activator. With the untreated bran the reduction in time was greater for Chiefkan than for Tenmarq. The bran extract had a different effect than the extracted bran. The nil effect or increase in time when the activator was used with Chiefkan bran extract indicates that this contained no substance which could be activated or that it even contains an inhibitor. Thus these varieties behave differently both towards the activator and towards the protease pepsin.

### Effect of Concentration of HCl at the Water-Dough Interface

That protease activity is influenced by the hydrogen-ion concentration of the solution is well known. Since the disintegration of the doughball starts at the water-dough interface, the time should be influenced by that pH which is near optimum for the protease. To try the influence of various concentrations of HCl on time, different amounts of a 0.1N solution of HCl were added to the water in the glasses. The results of combinations of acid and water in the glasses are shown in Table XI.

TABLE XI  
EFFECT OF THE CONCENTRATION OF HCl AT THE DOUGH-WATER INTERFACE

Combination	Tenmarq	Chiefkan
	<i>Min.</i>	<i>Min.</i>
200 c.c. water	121	59
190 c.c. water+10 c.c. 0.1N HCl	100	49
180 c.c. water+20 c.c. 0.1N HCl	59	41
160 c.c. water+40 c.c. 0.1N HCl	46	43

It is very evident that the concentration of HCl at the dough-water interface has a distinct effect on the time and that the differences between the two wheats tends to disappear as the acidity increases. Whether this was due to protease stimulation or to the action of the acid in weakening the gluten cannot be determined from this trial.

### Summary

The presence of wheat germ has a significant influence on "time." The time was shortened: (1) by adding to flour a mill stream rich in germ; (2) by the presence of germ in mill streams such as tailings and

sizings; and (3) by doubling the germ content of the meal. The time was lengthened by removing the germs from the kernels before these were ground into a meal.

The addition of bran or shorts increased the time on flour from both a short and a long "time" wheat. When the bran was extracted with water, it increased the time on a long but not on a short "time" wheat. The water extract of bran decreased the time on both wheats. While a finer granulation has a lengthening effect on time, this alone does not explain the longer time on mixtures of flour and bran.

When pepsin was added to mixtures of flour and untreated bran, to water-extracted bran, and to bran extract respectively, the time was shorter than on flour alone, and there were no significant differences between the long and the short "time" wheat flours.

When the activator cysteine-monohydrochloride was added to the mixture of flour and bran, both natural and water extracted, the time was shortened, but not as much as with pepsin. When added with the bran extract, this activator shortened the time of a long "time" but not of a short "time" wheat flour. In the case of the latter, the time was increased.

The time was shortened by adding HCl to the water in the glasses so as to increase the hydrogen-ion concentration at the dough-water interface. Since the decrease was greater for the long-time than for the short-time wheat, the differences between the two tend to disappear.

It seems from this and previous investigations that the time is influenced by at least four factors: proteases, protease inhibitors and activators, and gluten quality. These factors may remove the differences between long and short "time" wheats. However, even after some treatments differences still persist. Further investigations are in progress.

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## ABSORPTION-MOBILITY RELATIONSHIPS IN WHEAT-FLOUR DOUGHS<sup>1</sup>

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In earlier discussions of the "viscosity," or its reciprocal, the mobility of dough, it has been intimated at times that the proportion of water for the production of optimum quality of bread could be determined directly from such mobility measurements. Thus it has been suggested that doughs mixed to a certain definite mobility, as indicated by such an instrument as the farinograph, would then contain the requisite proportion of water for baking, regardless of the class, grade, and composition of the flour used in producing the dough. In order to test this hypothesis a series of studies were undertaken with flours of widely varying composition.

Nine flours falling into the three classes of so-called "strong," "medium strength," and "weak" were used. The "strong" flours were milled from hard red spring wheat, two of them being bakers' patents, the third a clear-grade flour. The "medium strength" flours included a spring wheat bakers' patent, a southwestern hard red winter wheat bakers' patent, and a southwestern hard red winter wheat bakers' patent of the 1935 crop. The "weak" flours were soft red winter wheat cake flours, two of which were short patents, while the third was of longer extraction. Their description and crude protein content are recorded in Table I.

Mobility was measured with a Brabender farinograph in consistency units, using 300 g. of the flour and sufficient distilled water to equal the particular absorption desired.

The Hobart-Swanson dough mixer was used in these tests. For determining the optimum absorption the doughs were mixed two minutes. The dough formula included 200 g. flour, 2 g. salt, 5 g. sugar, 6 g. yeast, and sufficient distilled water to equal the absorption desired. The doughs were brought out of the mixer at 30°C. Immediately on removal from the mixer they were folded in the hands ten times to insure that any inequalities of mixing, if present, would be more evenly distributed throughout the doughs. The doughs were then scaled into two portions of 160 grams each. This practice was followed for several reasons. In commercial practice a flour is judged by a loaf from a

<sup>1</sup> Journal Series Paper No. 1652, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul.

TABLE I  
DESCRIPTION OF THE NINE FLOURS

Biochem. No.	Flour	Crude protein (N×5.7)
		%
	<i>Weak Flours</i>	
17890	Soft red winter wheat flour, short extraction	8.30
17891	Soft red winter wheat flour, longer extraction	9.58
17934	Soft red winter wheat flour, short extraction	8.30
	<i>Medium-Strength Flours</i>	
17892	Spring wheat, bakers' patent	13.18
17893	Southwestern hard red winter wheat, bakers' patent	12.65
17894	Southwestern hard red winter wheat, bakers' patent 1935	11.48
	<i>Strong Flours</i>	
17895	Bakers' spring wheat clear	15.10
17896	Bakers' spring wheat patent	15.08
17935	Bakers' spring wheat patent	15.10

scaled weight of dough, not from a scaled weight of flour. It also avoids inequalities due to unequal-sized doughs as absorption is varied.

Each piece was folded in the hands ten times to round it up and provide it with a smooth, tight, surface film. The doughs were fermented in porcelain-coated, round-bottom bowls which were lightly greased with a hydrogenated shortening to make handling of the doughs during punching more uniform. Were all doughs mixed to the same degree of mobility this would not be necessary, but, where mobility varies from extremely soft and sticky to very stiff doughs, an error would be introduced because of unequal handling. This error, reflected in the loaf, should not be charged to absorption but to handling, and the procedure followed was designed to reduce this error to a minimum.

Fermentation was conducted in a thermostat provided with humidity and temperature control. Weak flour doughs were fermented 1½ hours with a punch of ten folds one hour after mixing. Thirty minutes later they were hand-molded on a piece of heavy canvas belting. A small quantity of dusting flour was used to prevent the doughs from sticking to the belting. Doughs made of "medium strength" and "strong" flours were fermented three hours. They were punched with fifteen folds by hand after 105 minutes of fermentation and again with ten folds after 50 minutes of fermentation following the first punch. In 25 minutes they were hand-molded as previously described for "weak" doughs and all were panned in lightly greased, high-sided, black iron pans.

All doughs were proofed for 55 minutes at 30°C. in the fermentation cabinet and then baked for 25 minutes at 230°C. in an automatically

controlled, electric oven on revolving platforms. Humidity in the oven was supplied by an open bowl of water at the level of the rotating platforms.

The loaf type, according to the Blish method, was assigned each loaf as well as a score on a zero-to-ten basis. Thirty minutes after removal of the loaf from the oven its volume was determined by means of the Werner loaf-measuring apparatus. On the following day the loaves were cut open and scored for grain and texture. Quality score (Q.S.) was calculated by the following formula:  $Q.S. = 0.1 (L.V. - 200) + (L.T.S. + 2G + T)$ .

L.V. = loaf volume in cc.

200 represents average original volume of dough in cc. when panned

L.T.S. = loaf type score

G = score for grain

T = score for texture

The approximate absorption of each flour was predetermined by means of the Brabender farinograph. A charge of 300 grams of flour and enough distilled water to bring the peak of the development curve to 550 units of consistency were first used. With this determination as a guide, the baking of the samples proceeded as previously described, the absorption being varied by one percent intervals. Doughs ranged in mobility from very stiff to very soft, or until loaf volumes showed an increase from low, through a maximum, and then to a definitely lower figure.

After the flours had all been baked and Q.S. or quality score calculated, a curve was plotted for each flour with Q.S. on the vertical axis and absorption on the horizontal axis. These curves are shown in Figure 1. It will be noted that "medium strength"

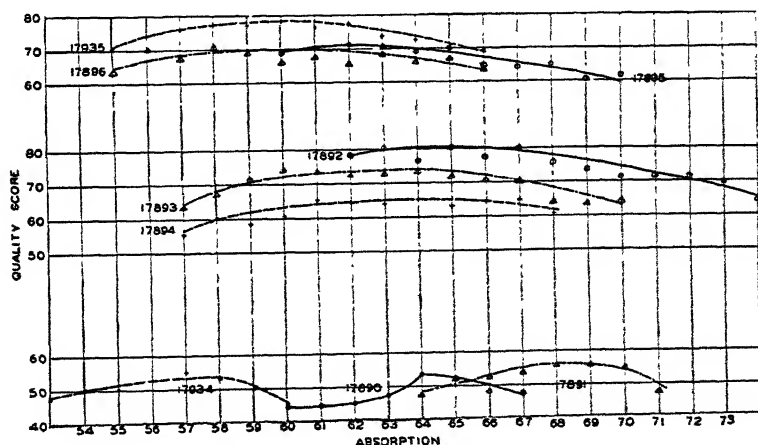


Fig. 1. Relationship of absorption to quality score.

and "strong" flours show no single definite peak such as is shown by the "weak" flours. In such cases an absorption was chosen, arbitrarily, as the optimum, from near the middle of the high part of the curve. As might be expected, these curves show that absorption is not an extremely critical factor in producing bread from strong bread flours. Such is not true when the "weak" flours are considered.

After the optimum absorption on each flour had thus been determined, the mobility curves of each were recorded on the farinograph for a 30-minute period. The absorptions used in these mobility studies were the optimum as determined by baking and one and two percent below and above the optimum, five levels in all. The effect of increments of one percent absorption on the mobility of the flour being studied and the different effects on flours of different "strengths" were thus disclosed. These data are recorded in Table II.

Following the mobility studies, using the farinograph, the flours were again baked, using the optimum absorption previously determined by the preliminary baking. In this second baking study the procedure was varied by the use of four periods of mixing time, 1, 2, 3, and 5 minutes, respectively. Each dough was scaled into 160-gram portions and three such portions were fermented for  $1\frac{1}{2}$ ,  $2\frac{1}{4}$ , and 3 hours, respectively. Thus each flour was accorded three fermentation treatments with each of the four states of mobility as imposed by the Hobart-Swanson mixer.

The purpose of this second study of the nine flours, by baking methods previously described, was to transfer the physical properties of the doughs at different stages of mechanical development in the farinograph to a form, the baked loaf, where the effect of these physical properties on loaf volume, loaf type, grain, texture, and loaf quality could be accurately measured. These stages of mixing development are equivalent to 6, 12, 18, and 30 minutes respectively in the farinograph.

From a study of the measurements of loaf volume and calculations of loaf-quality scores on these twelve loaves in comparison with the farinograph curves showing rate and degree of development (see Table III) and time, rate, and degree of slackening of dough, one is able to determine whether the information given by the farinograph alone is sufficiently exact and accurate for the purpose.

TABLE II  
EFFECTS OF ABSORPTION ON MOBILITY

Flour	Absorption	Time to peak	Consistency units at peak of farinograms	Average difference in consistency units per 1% water	Consistency units after 30 min. mixing	Average difference in consistency units per 1% absorption
	%	min.				
17935	62	7½	670		490	
"	61	7	710		510	
"	60	6½	730		525	
"	59	6	770		535	
"	58	5	810	35	550	15
17896	63	6½	690		505	
"	62	6	710		510	
"	61	6	750		530	
"	60	4½	800		550	
"	59	4½	850	40	565	15
17895	64	6	610		425	
"	63	5	645		440	
"	62	4½	690		460	
"	61	5	725		470	
"	60	4½	760	39	485	15
17894	66	12	500		405	
"	65	11¾	515		420	
"	64	6	550		430	
"	63	5	570		450	
"	62	5	600	25	465	15
17893	66	10½	540		400	
"	65	10	550		410	
"	64	10½	570		420	
"	63	6½	605		445	
"	62	6	620	20	450	12½
17892	67	11½	455		340	
"	66	11	470		345	
"	65	11¾	510		370	
"	64	12	530		400	
"	63	6	580	31	420	20
17934	59	8	350		270	
"	58	8½	370		290	
"	57	8¾	390		305	
"	56	9	400		320	
"	55	9½	420	17½	335	16
17891	70	6½	325		220	
"	69	6	340		220	
"	68	5¾	355		225	
"	67	6	420		240	
"	66	6	425	25	280	15
17890	66	6	340		235	
"	65	6	340		230	
"	64	6	365		240	
"	63	6½	410		250	
"	62	7	425	22½	280	11



TABLE III  
LOAF QUALITY SCORES

Flour No.	Fermentation periods—hours											
	1½				2¼				3			
	1	Minutes mixed			1	Minutes mixed			1	Minutes mixed		
	2	3	5		2	3	5		2	3	5	
17895	63.5	68.5	71.7	64.0	60.5	70.2	65.7	53.2	60.0	67.5	68.0	54.2
17896	79.5	80.5	72.0	64.5	75.0	73.7	67.5	52.7	66.7	68.7	62.0	52.7
17935	84.2	85.0	86.0	69.5	86.0	78.0	70.0	56.5	77.5	75.5	64.7	55.7
17892	66.2	77.5	79.0	73.2	63.2	65.5	66.7	62.0	57.7	64.7	63.5	60.2
17893	71.7	77.5	62.2	69.5	65.5	66.2	66.7	64.5	58.2	64.7	63.2	60.0
17894	64.7	59.7	69.5	64.7	58.0	61.0	63.5	61.5	57.0	60.5	65.7	62.0
17890	46.7	46.7	55.0	50.5	40.0	41.0	46.0	41.5	37.2	40.0	40.5	43.5
17891	43.0	45.5	52.5	50.0	42.5	45.0	45.7	43.0	39.0	40.5	42.2	40.7
17934	48.0	50.7	51.7	52.0	41.0	46.7	46.7	45.0	40.0	43.0	42.5	41.5

### Discussion

If doughs prepared with the nine flours were mixed to a uniform consistency of 550 farinograph units, the proportion of water recorded in the second column of Table IV would be required. As a matter of fact, the optimum absorption, as determined by baking tests, was often

TABLE IV  
ABSORPTIONS DETERMINED BY BAKING TESTS VERSUS ABSORPTIONS AS DETERMINED BY "STANDARD CONSISTENCY" METHODS

Biochem. No.	Absorption at 550 consistency units	Optimum absorption (by baking)	Consistency units at optimum absorption
	%	%	
17890	59	64	365
17891	61.3	68	355
17934	55	58	390
17892	66	65	510
17893	66.7	64	570
17894	65.7	64	550
17895	71	62	690
17896	71	61	750
17935	71	60	730

quite different from that required to produce dough of a standard consistency at the time of the original mixing. This is evident on comparing the data in column 3 of Table IV with those in column 2. It accordingly followed that the consistencies of doughs which baked best

when prepared from the nine flours were not uniform, as is evident from the data recorded in column 4 of the same table. The "weak" flours had low absorptions at 550 consistency farinograph units, the "medium strength" flours had a higher absorption, approximately 66%, and the "strong" flours had a very high absorption of 71%. This is in general agreement with the commercial practice of assigning high absorptions to "strong" flours and low absorptions to "weak" flours.

The "optimum baking quality" doughs prepared from "weak" flours were found to have an average absorption of 63.3% instead of 58.4% based upon the farinograph consistency, a gain of almost 5%; "medium strength" flours 64.7%, instead of 66.8%, a loss of about 2%; and "strong" flours 61% instead of 71%, a loss of 10%. Thus the fallacy of using a single mobility value for a wide range of flours in determining their absorption by the farinograph is apparent.

The second mobility studies, using the farinograph, with the optimum absorption of each flour, showed that mobility at optimum absorption is, on the average, approximately 370 for "weak" flours, 543 for "medium" flours, and 723 for "strong" flours. In the same class of flours, the longer-extraction flours had greater mobility or lower consistency-unit value at optimum absorption than the shorter extraction flours.

### Summary and Conclusions

The relationship between optimum absorption and mobility as measured with the farinograph at that absorption was determined on nine flours, three of which were in each of "weak," "medium strength," and "strong" classes.

It was apparent that the mobility of a flour at optimum absorption is characteristic of that particular flour and that it varies widely between flours differing considerably in "strength."

# FURTHER STUDIES UPON THE RELATIVE MACARONI- MAKING QUALITY OF A NUMBER OF DURUM WHEAT VARIETIES<sup>1</sup>

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The present paper represents an extension of the studies upon the quality of durum wheat varieties initiated in 1934 by Binnington and Geddes (1937). Since that time, certain additional quality tests have been developed and a more extensive range of samples studied. It has thus been found feasible to draw more definite conclusions than were possible with the limited number of samples available in the earlier studies. The accumulation of a larger volume of data has also made possible statistical examination of the relations between certain of the more important quality factors.

As the authors have previously indicated, the literature in this field is extremely meager, being confined largely to a brief description of the agronomic, milling, and macaroni-making characteristics of the standard varieties contained in the "Dictionary of Spring Wheat Varieties" published by the Northwest Crop Improvement Association (1933). More recently, however, Fifield *et al.* (1937) have published the results of studies extending over a five-year period, which represent a valuable contribution to our knowledge of this subject.

## Materials and Methods

The wheats employed in these studies were all experimentally grown on one-sixtieth-acre plots during the years 1935, 1936, and 1937. In 1935, samples were available from Morden, Manitoba, only; in 1936, Morden, Brandon, and Winnipeg; and in 1937 these three stations were supplemented by Melita, Manitoba, and Indian Head, Saskatchewan. Official grades and weights per Imperial bushel were secured upon all samples, which were milled into semolina and processed into macaroni by the technique outlined by Binnington and Geddes (1936). Protein and carotene determinations were made on the wheats and semolinas by the official A.A.C.C. methods, using the spectrophotometric procedure for the carotene determinations; water-saturated n-butyl alcohol, as described by Binnington, Sibbitt and Geddes (1938) was employed

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as a solvent for the later groups of samples. Where results were secured by use of the older naphtha-alcohol solvent, conversion to a butyl-alcohol basis has been made by use of the formulas outlined by Binnington and Geddes (1939). All samples for protein and pigment determinations were prepared by grinding in a Wiley mill fitted with a  $\frac{1}{2}$ -mm. sieve, and all results are expressed on a 13.5% moisture basis.

Breaking strength of macaroni and tenderness of the cooked product were measured by the procedures outlined by Binnington, Johansson, and Geddes (1939), and color analyses of macaroni were also conducted by the methods described by these investigators, employing a modified Bausch and Lomb Type H. S. B. Color Analyzer with suitable Munsell discs.

### Experimental Results

In view of the large number of samples studied, individual data are not presented in all cases, and the results obtained have accordingly been summarized by years. These results are detailed in Tables I, II, and III, and a general summary of means for all years and stations is given in Table IV. The listing of the samples is in the order of decreasing mean color score as shown in Table IV.

Macaroni color may be justifiably considered as the major measurable quality characteristic, and experience gained in this laboratory indicates that the so-called "computed" or "single figure" color score is a very reliable quantitative index of visual color. It is a difficult matter to establish rigid limits for this value, however, as the general level of durum wheat quality varies widely from year to year, depending upon the magnitude and nature of the degrading factors present, and a value that would be considered as normal for one crop may not be attainable at all in the following year. In a general way, however, a level of 18 units for this value would appear to represent a normal lower limit. In years where the general color level is low, this might be reduced to 17.5 units, but samples ranking below this value are definitely unsatisfactory.

Utilizing the above values as criteria, an inspection of the data in Tables III and IV indicates that only three varieties, namely Arnautka, Mindum, and Akrona, can be considered as consistently yielding macaroni of satisfactory commercial quality over a period of years; the latter variety can only be classed as "borderline." This conclusion is confirmed by an examination of the color analysis data for the individual stations presented in Table V. Using 18 units of color score as the critical level, seven out of eight samples of Arnautka were above this value, Mindum five out of eight, and Akrona, Pelissier, and Nodak

TABLE I

WEIGHT PER BUSHEL, GRADE, AND SEMOLINA YIELD  
Comparison of Mean Values—All Stations—Years 1935, 1936, and 1937

Variety	Weight per Imperial bushel (cleaned wheat)			Official grade <sup>1</sup>			Semolina yield		
	1935	1936	1937	1935	1936	1937	1935	1936	1937
	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>				<i>%</i>	<i>%</i>	<i>%</i>
Arnautka	59.0	62.5	63.2	4.0	3.0	2.4	26.4	30.4	31.2
Mindum	61.0	62.7	64.0	3.0	3.0	2.0	27.4	30.3	34.8
Akrona	56.0	62.3	63.9	4.0	3.3	2.4	24.5	29.1	32.0
Iumillo	—	62.6	63.9	Red	Red	Red	—	29.3	28.5
Pelissier	57.8	61.3	62.7	4.0	3.3	2.6	26.0	29.7	31.7
Monad	60.8	62.7	64.3	5.0	3.3	3.0	27.0	29.8	30.9
Nodak	60.2	62.8	64.2	4.0	3.0	2.6	29.0	30.1	31.9
Kubanka	—	62.8	63.4	—	3.0	2.2	—	30.7	31.6
Acme	60.0	62.5	64.4	4.0	3.0	3.0	28.6	29.8	31.5
Pentad	—	63.2	64.5	Red	Red	Red	—	29.3	28.5
Golden Ball	57.0	61.2	61.4	5.0	4.3	3.6	24.8	29.7	29.8

<sup>1</sup> In computing the mean values, grades 1 C.W., 2 C.W., 3 C.W., 4 C.W., and 5 C.W. were assigned numerical values of 1, 2, 3, 4, and 5, respectively.

TABLE II

PROTEIN AND CAROTENE DATA <sup>1</sup>  
Comparison of Mean Values—All Stations—Years 1935, 1936, and 1937

Variety	Protein content						Carotene content					
	Wheat			Semolina			Wheat			Semolina		
	1935	1936	1937	1935	1936	1937	1935	1936	1937	1935	1936	1937
	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>ppm.</i>	<i>ppm.</i>	<i>ppm.</i>	<i>ppm.</i>	<i>ppm.</i>	<i>ppm.</i>
Arnautka	15.3	17.0	14.8	14.1	15.6	13.2	6.76	4.73	5.69	5.87	3.94	4.02
Mindum	14.3	17.3	14.1	13.2	15.7	12.8	5.79	4.56	6.23	6.39	3.62	4.60
Akrona	14.3	17.9	13.9	13.2	16.6	12.5	7.74	5.03	6.25	7.96	4.28	5.09
Iumillo	—	17.4	15.3	—	15.9	13.5	—	4.15	4.91	—	3.89	3.66
Pelissier	14.6	16.2	13.9	13.2	14.8	12.7	5.29	3.98	5.76	5.85	3.42	4.44
Monad	14.8	17.2	14.2	13.3	15.3	12.3	4.79	3.91	4.42	4.11	3.12	3.42
Nodak	14.3	16.3	14.0	12.8	14.7	12.3	5.63	4.09	5.01	5.35	3.48	3.82
Kubanka	—	19.6	14.1	—	14.7	12.5	—	4.18	5.13	—	2.22	3.94
Acme	13.8	16.6	14.3	12.6	14.5	12.5	4.62	3.57	4.39	4.19	2.99	3.21
Pentad	—	17.6	14.6	—	15.8	12.9	—	4.04	4.63	—	3.13	3.32
Golden Ball	16.7	16.7	14.8	14.4	14.2	12.6	5.62	5.18	6.86	5.39	3.81	5.08

<sup>1</sup> Results expressed on a 13.5% moisture basis.

TABLE III

BREAKING STRENGTH, TENDERNESS SCORE, AND COLOR SCORE OF MACARONI  
Comparison of Mean Values—All Stations—Years 1935, 1936, and 1937

Variety	Breaking strength (arbitrary units)		Tenderness score (arbitrary units)			Computed color score		
	1936	1937	1935	1936	1937	1935	1936	1937
Arnautka	136	175	108.4	127.3	110.0	23.7	16.7	19.3
Mindum	158	177	99.3	98.1	113.4	24.4	17.7	18.4
Akrona	143	169	124.6	111.6	111.3	17.8	18.1	17.8
Iumillo	155	191	—	—	120.6	—	16.7	17.7
Pelissier	149	185	—	—	117.2	17.5	17.1	17.0
Monad	161	194	—	110.1	111.3	17.8	16.6	17.1
Nodak	157	183	—	—	117.0	16.8	16.7	16.9
Kubanka	163	185	—	—	119.1	—	15.6	16.7
Acme	168	198	—	—	113.1	16.0	15.4	16.5
Pentad	162	190	—	—	126.1	—	15.3	16.1
Golden Ball	138	179	—	—	116.6	15.7	16.0	15.8

TABLE IV

SUMMARY OF MEAN VALUES—ALL STATIONS—ALL YEARS<sup>1</sup>

Variety	Weight per im- perial bushel (cleaned wheat)	Official grade	Semo- lina yield	Protein <sup>2</sup>		Carotene <sup>2</sup>		Macaroni		
				Wheat	Semo- lina	Wheat	Semo- lina	Break- ing strength	Ten- der- ness score	Com- puted color score
	<i>lbs.</i>		<i>%</i> <sup>*</sup>	<i>%</i>	<i>%</i>	<i>ppm.</i>	<i>ppm.</i>	<i>Arbi- trary units</i>	<i>Arbi- trary units</i>	
Arnautka	62.5	3.1	30.1	15.6	14.1	5.48	4.20	166	112.5	19.2
Mindum	63.2	2.7	31.5	15.2	13.8	5.62	4.49	170	107.0	19.0
Akrona	62.4	3.2	30.2	15.3	13.9	6.00	5.13	158	113.6	17.9
Iumillo	63.4	Red durum	28.8	16.2	14.3	4.63	3.62	176	120.2	17.3
Pelissier	61.7	3.3	30.3	14.7	13.6	5.10	3.99	169	117.2	17.1
Monad	63.3	3.8	30.1	15.2	13.4	4.29	3.40	180	111.0	17.0
Nodak	63.6	3.2	31.0	14.8	13.2	4.78	3.88	171	117.3	16.8
Kubanka	63.0	2.6	31.2	15.0	13.3	4.77	3.85	176	119.0	16.3
Acme	63.2	3.3	30.6	15.0	13.2	4.15	3.14	185	113.0	16.1
Pentad	64.0	Red durum	28.8	15.7	13.9	4.41	3.25	176	126.0	15.8
Golden Ball	60.8	4.2	28.0	15.6	13.3	6.16	4.70	162	116.5	15.8

<sup>1</sup> Varieties arranged in order of mean color scores.

<sup>2</sup> Results expressed on a 13.5% moisture basis.

TABLE V  
COMPARISON OF INDIVIDUAL COLOR SCORE DATA

Variety	Morden			Brandon		Winnipeg		Melita	In- dian Head
	1935	1936	1937	1936	1937	1936	1937	1937	1937
Arnautka	23.7	—	19.3	18.3	20.0	15.1	18.0	19.7	19.6
Mindum	24.4	16.8	19.4	18.6	19.4	—	16.8	17.1	19.3
Akrona	17.8	17.5	18.7	17.8	17.6	18.9	17.3	16.7	18.8
Iumillo	—	15.2	19.6	18.1	19.0	16.8	15.4	16.7	17.6
Pelissier	17.5	16.7	18.0	18.0	18.7	16.7	15.6	15.2	17.6
Monad	17.8	16.2	17.5	17.0	17.3	16.6	15.6	17.5	17.4
Nodak	16.8	16.5	19.2	18.0	18.2	15.6	15.3	14.6	17.4
Kubanka	—	15.5	18.2	16.1	18.2	15.1	15.7	14.3	17.2
Acme	16.0	16.2	17.6	16.3	18.0	13.8	14.5	15.6	16.6
Pentad	—	14.9	16.7	16.2	16.4	14.9	15.1	—	16.2
Golden Ball	15.7	15.8	17.5	16.4	16.4	15.7	15.3	12.5	17.3

each only three out of eight. An item of some interest is the relatively high placing accorded to Iumillo. On a visual basis, this variety would be placed close to the bottom of the list, because of the noticeable presence of bran specks in the macaroni. The red durum, Iumillo and Pentad, were included in the tests only to ascertain their value as parents in the production of high-quality rust-resistant hybrids, and it would appear that Iumillo possesses far better characteristics in this regard than Pentad. This conclusion has been borne out experimentally; of a number of rust-resistant hybrids examined in recent years, all the promising lines have originated from Iumillo-Mindum crosses.

The low placing of Kubanka is rather striking, as this variety is usually classed commercially along with Mindum as representing the two most satisfactory varieties. In the studies previously reported by Binnington and Geddes (1937), it was indicated that the sample of Kubanka employed was grown from a pure-line selection and was probably not typical of the variety as commercially grown. In the later studies reported here, representative commercial Kubanka seed was used and the results are accordingly free from this criticism. It is possible, as Fifield *et al.* (1937) point out, that this variety may be affected to a greater extent by unfavorable growing conditions than Mindum, but whatever the reason may be, it would appear to be a definitely undesirable variety for the durum-growing regions of western Canada.

Passing from the question of macaroni color to consideration of the other factors involved, it will be noted that for all stations and years weight per bushel is maintained at a fairly uniform level for most of the varieties. Test weight varies with environmental conditions

as shown by the mean values for each year and was at a generally low level in 1935, as a result of heavy stem-rust infection; within each year, however, Golden Ball and Pelissier show a definite tendency to low test weight. The yield of semolina is related to the weight per bushel, excepting in the case of the red durums. It must be emphasized that the experimental yields are low in comparison with those obtained in commercial mills, for the reason that the primary object is to secure a semolina essentially similar to the commercial product; with the short experimental milling system, this can only be accomplished at the expense of yield.

Protein content of the wheat is also greatly influenced by environmental factors but would appear also to be influenced to some extent by variety, Pelissier and Nodak tending to exhibit the lowest values and Golden Ball the highest. Wheat carotene content, however, is definitely a varietal characteristic, being lowest in Acme and highest in Golden Ball. Neither of these factors individually, however, is closely associated with macaroni color score.

The remaining macaroni-quality factors, transverse breaking strength and tenderness score, show essentially similar mean values regardless of variety, although Mindum appears to produce macaroni falling below the general level of tenderness.

A detailed examination of the results secured in the past four years suggested the possibility of some associations existing between certain of the properties studied, and the data were therefore submitted to statistical analysis; the results obtained are worthy of brief discussion.

### Inter-varietal Relations between Wheat Protein, Semolina Protein, and Tenderness of Macaroni

Intra-station, inter-varietal correlations between wheat protein, semolina protein, and macaroni tenderness were computed for the 1937 crop data for four of the stations represented. From the results recorded in Table VI it will be noted that while there is a high correlation be-

TABLE VI  
INTER-VARIETAL CORRELATIONS BETWEEN WHEAT PROTEIN, SEMOLINA PROTEIN, AND MACARONI TENDERNES

Correlation between	Correlation coefficients <sup>1</sup>			
	Winnipeg	Brandon	Melita	Indian Head
Wheat protein and semolina protein	.900	.863	.977	.965
Wheat protein and tenderness score	-.015	.317	.423	.205
Semolina protein and tenderness score	-.016	.233	.337	.237

<sup>1</sup> Value of  $r$  at 5% pt. = .497.



tween wheat and semolina protein, the correlation between protein content and macaroni tenderness is not significant. These results suggest the possibility of marked inter-varietal variations in protein "quality."

### Inter-varietal Relations between Pigment Content of Wheat, Semolina, and Macaroni

Use of the new butyl-alcohol solvent has made possible estimation of the pigment content of ground macaroni, as this solvent releases appreciable amounts of pigment which could not formerly be extracted with naphtha-alcohol.

For this study the mean values, over all stations, for each of the varieties from the 1937 crop were employed, and the results are recorded in Table VII.

TABLE VII  
INTER-VARIETAL RELATIONS BETWEEN PIGMENT CONTENT OF WHEAT,  
SEMOLINA, AND MACARONI

Correlation between	Correlation coefficients <sup>1</sup>
Wheat carotene and semolina carotene	.426
Wheat carotene and macaroni carotene	.504
Semolina carotene and macaroni carotene	.585

<sup>1</sup> Value of  $r$  at 5% pt. = .217.

These correlations are lower than would be anticipated. Variations in the germ content of experimentally processed semolina might readily account for the relatively low relations between wheat and semolina carotene. While the use of *n*-butyl alcohol permits the recovery of larger quantities of pigment from macaroni than naphtha-alcohol, the values obtained with it rarely approach the corresponding semolina values, and variations in the residual unextracted carotene would influence the magnitude of the correlations between wheat and semolina carotene and macaroni carotene.

### Relations between Wheat and Semolina Protein, and Carotene and Macaroni Color Scores

In view of the magnitudes of the correlations cited above, and also the possible effects of protein content upon macaroni color through its relation to translucency and vitreousness, the series of correlations recorded in Table VIII were computed for the available data from the 1935 and 1936 crops, no attempt being made to classify the results

according to variety, grade, location, or crop year. The correlations found are given in Table VIII.

TABLE VIII  
RELATIONS BETWEEN WHEAT AND SEMOLINA PROTEIN, AND CAROTENE  
AND MACARONI COLOR SCORES

Correlation between	Correlation coefficients <sup>1</sup>
Wheat protein and wheat carotene	-.566
Wheat carotene and macaroni color score	.056
Wheat protein and macaroni color score	.025
Semolina protein and semolina carotene	-.562
Semolina protein and macaroni color score	.100
Semolina carotene and macaroni color score	.205

<sup>1</sup> Value of  $r$  at 5% pt. = .224.

These results suggest that no intra- or inter-varietal relations exist between the various properties with the exception of the negative correlations between protein and carotene.

In addition to the computations detailed above, a similar series of correlations was calculated for the 1936 crop data. Significant correlations were obtained only between: wheat and semolina protein,  $r = .871$ ; wheat and semolina carotene,  $r = .870$ ; and semolina carotene and *semolina* color score,  $r = .805$ . It should be pointed out that no inter-varietal relation exists between semolina color score and macaroni color score, and accordingly the correlation of 0.805 between semolina carotene and semolina color is of no utility in predicting macaroni color.

The principal result of the above statistical studies is to emphasize the fact that no single analytical factor can be relied upon for the inter-varietal prediction of macaroni quality, and the best index as yet available is the color of the finished product. It should be noted particularly that wheat carotene alone is valueless for the inter-varietal prediction of macaroni color.

### Summary

Ninety-nine samples of durum wheat, representing 11 standard varieties produced in western Canada during the years 1935, 1936, and 1937, have been milled into semolina, and macaroni has been produced therefrom. Mean values are presented for each year, covering the following analytical factors: official grades, weight per bushel, semolina yield, wheat and semolina protein and carotene, macaroni breaking strength, and tenderness and color scores.

Of the varieties studied, only Arnautka, Mindum, and Akrona have

been found to produce macaroni of satisfactory commercial quality with a reasonable degree of consistency over the three-year period.

Weight per bushel and semolina yield are governed chiefly by environmental factors, whereas protein and carotene are to a greater extent influenced by variety.

Significant positive inter-varietal correlations were obtained only between wheat protein and semolina protein, wheat carotene and macaroni carotene, and semolina carotene and macaroni carotene. Negative inter-varietal correlations were found between wheat protein and wheat carotene and between semolina protein and semolina carotene.

It is emphasized that macaroni quality cannot be predicted from any single analytical test as yet applied to the wheat; in particular, for inter-varietal predictions, wheat carotene alone is valueless as an index of macaroni color.

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# THE ANALYTICAL ERROR OF THE KJELDAHL NITROGEN TEST <sup>1</sup>

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The evaluation of analytical errors influencing the reliability of such important routine determinations of the cereal laboratory as moisture, protein, and ash has received considerable attention by the American Association of Cereal Chemists. For several years, the Committee on Methods of Analysis sponsored collaborative studies on these determinations, the results of which were subjected to statistical treatment by Treloar (1928, 1929, 1930, 1932, 1933). These studies clearly showed the existence of systematic errors between laboratories in addition to random errors of appreciable magnitude within laboratories. Assuming normality of the error distributions, error ranges that may be anticipated in extensive replication within laboratories were computed from the standard deviations of the replicate errors and practical permissible limits of accuracy set up.

Treloar (1932) has pointed out that one would expect a positive correlation between the magnitudes of the random error and of the constituent being determined, and that if such a correlation exists, standards of random error based on analyses of samples of widely varying protein content may be too lenient for some samples and too stringent for others. The A.A.C.C. collaborative studies were necessarily confined to a limited number of samples and it was thus impossible to test this hypothesis.

In our laboratory records, duplicate protein tests are available for several thousand samples of wheat covering a wide range in protein content and representing all grades of Western Canadian hard red spring wheat. A similar series of flours, but smaller in number, has also been tested. It seemed of interest to use these data for a further statistical study of the analytical errors of the Kjeldahl nitrogen test.

The Kjeldahl nitrogen test is conducted in this laboratory essentially as outlined in Cereal Laboratory Methods (American Association of Cereal Chemists, 1935) with certain modifications for convenience and speed. Both mercuric oxide and copper are employed as catalysts, sodium thiosulphate is used as a mercury precipitant, and 0.06265N  $\text{H}_2\text{SO}_4$  and NaOH are used, the former being adjusted to correct for

<sup>1</sup> Contribution from the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba. Published as paper No. 152 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.

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the blank. Inversely calibrated burettes are employed for back-titration so that the reading divided by 2 gives the percentage of protein directly. The tests are run in batches of 24; the digestion heating units are all standardized to a capacity of 570 watts and the distillation heating units to 800 watts; 40 to 45 minutes are allowed for digestion, while the distillation requires 20 to 25 minutes. Wheat samples (approximately 60 g.) are ground to a flour-like consistency in a Hobart burr mill, placed in ointment tins, and duplicate one-gram portions weighed from the same grind.

### Results

The protein data available for statistical study comprised duplicate tests on 10,988 samples of wheat and 1,482 samples of flour; the range in protein content for wheat was 7.0% to 21.0% and for flour 7.0% to 18.0%. In classifying the data, a frequency table was compiled in which the differences between duplicates in 0.03% intervals were tabulated for each 1% increment in mean protein content.

Casual inspection of the frequency distributions given in Tables I and II reveals that, contrary to expectation, there is no relation between protein content and the magnitude of the error; the correlation coefficient was computed for the wheat errors and found to be  $r = 0.056$  (5% point = 0.019). In view of this low correlation, the entire series of data can be combined in order to study the errors in protein determinations at all levels; these are graphically represented in Figures 1 and 2 for wheat and flour respectively.

Curve type was measured by application of R. A. Fisher's "k" statistics as outlined by Goulden (1936), in which two statistics,  $g_1$  and  $g_2$ , are computed;  $g_1$  is a measure of symmetry; a symmetrical curves gives a  $g$  value of zero while positive and negative values indicate positive and negative skewness. A positive value of  $g_2$  indicates a peaked or leptokurtic curve, and a negative value a flat-topped or platykurtic curve. As would be expected,  $g_1 = 0$ , since theoretically one-half the errors would be considered positive and the other half negative; the  $g_2$  values, however, for both wheat and flour were positive and highly significant and, hence, the error distributions are leptokurtic or peaked. In such curves, the center is higher and more pointed than normal and the tails are extended.

The explanation of leptokurtic distributions under routine conditions appears to lie in the occurrence of occasional gross discrepancies attributable to unnoticed accidents rather than ordinary experimental errors in an otherwise approximately normal system. In this connection, it is of interest to mention the results of an unpublished statistical study

TABLE I  
DISTRIBUTION OF WHEAT-PROTEIN ANALYTICAL ERRORS CLASSIFIED ACCORDING TO PROTEIN CONTENT

Difference between duplicates	Protein Content																
	7.0-7.9	8.0-8.9	9.0-9.9	10.0-10.9	11.0-11.9	12.0-12.9	13.0-13.9	14.0-14.9	15.0-15.9	16.0-16.9	17.0-17.9	18.0-18.9	19.0-19.9	20.0-20.9	21.0-21.9	Total	
%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
00-02	4	9	18	28	114	236	268	414	248	90	69	25	19	9	1	1,552	14.11
03-05	2	9	25	45	128	242	279	474	253	104	45	10	4	1	1	1,522	14.76
06-08		6	22	37	79	199	214	324	211	81	20	6	1	2		1,194	10.87
09-11		8	15	29	123	251	300	542	267	114	36	10	7	7	1	1,718	15.64
12-14	1	2	10	18	60	138	161	256	157	72	21	5	1	1		900	8.19
15-17	1	3	7	15	66	155	165	337	199	71	17	10	25	23	2	1,049	9.55
18-20	1	2	6	12	71	135	181	323	159	77	40	22	25	23	2	1,079	9.82
21-23			3	9	23	81	120	203	133	33	9	9	2			615	5.60
24-26				2	11	42	64	86	62	34	9	2	2			320	2.91
27-29		1		2	1	6	26	41	39	13	7	1		1	1	137	1.23
30-32		1		2	2	45	25	40	45	16	9	3				179	1.62
33-35				3	3	5	13	19	20	6	4	1		2		67	0.61
36-38					3	6	13	15	15	6	4	1				73	0.66
39-41					4	3	11	18	14	7	3	2				96	0.87
42-44				1	2	2	14	16	17	3	1	2			1	25	0.23
45-47		1				9	10	14	14	4	2					53	0.48
48-50			1			3	4	4	10	9	2	2	1	1		38	0.35
51-53						1	2	5	7	1	1					17	0.15
54-56						1	1	4	3	1	2		1			12	0.11
57-59				1	2	2	4	2	1	2	1					15	0.14
60-62						2	1	4	3	1	2	1				10	0.09
63-65						1	1	1	1	1	2	1		1		5	0.05
66-68						1	1	1	2	1	1	1				21	0.19
69-71						1	5	8	2	1	1					10	0.09
72-74						2	2	3	4	1	1					12	0.11
75-77						1	2	3	4	1	1					58	0.53
78-80				1	8	10	10	8	7	5	3	2	1			35	0.32
81-83			2	1	2	3	13	10	5	2						17	0.15
84-86					1	6	6	4	3	1						31	0.28
87-89					2	—	—	4	8	—						14	0.13
90-92		1	3		1	—	—	10	1	3	1					9	0.08
93-95		1	1	2	1	—	—	4	8	—						9	0.08
96-98			1	3	1	—	—	1	1	2	1					4	0.04
Total:	9	46	116	217	708	1,589	1,913	3,186	1,907	761	312	106	62	49	7	10,988	100.00
Number Per cent	0.08	0.42	1.06	1.97	6.44	14.46	17.41	29.00	17.36	6.93	2.84	0.96	0.56	0.45	0.06		



by J. W. Hopkins, Division of Biology and Agriculture, National Research Council of Canada, of a series of duplicate loaf volumes obtained in several Canadian cereal chemical laboratories co-operating in the work of the Associate Committee on Grain Research; the loaf volume deviations in all laboratories and by all formulas showed a definite leptokurtic tendency. In his confidential report to the co-operating laboratories, Hopkins pointed out that with such distributions the rejection of outlying observations may be expected to result in a normal distribution of enhanced precision and thus to improve the accuracy of the mean.

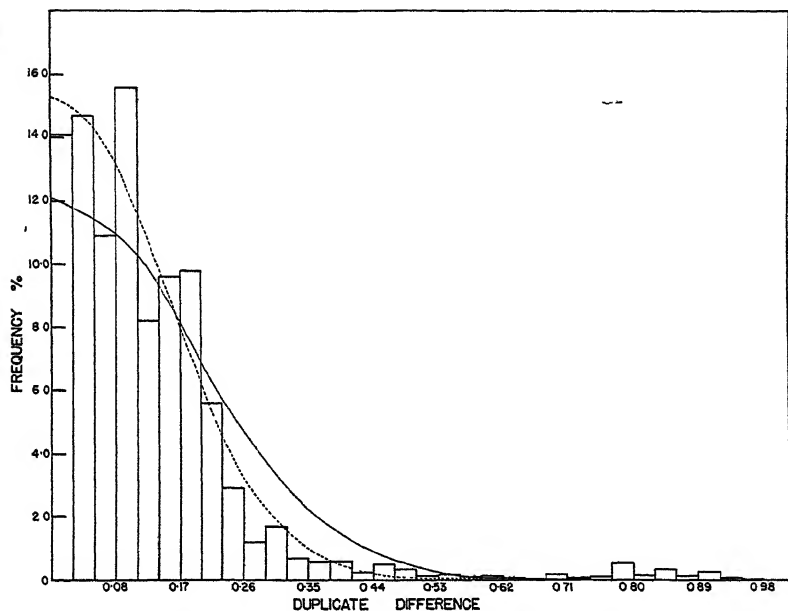


Fig. 1. Percentage frequency distribution of differences between duplicates for protein content of 10,988 wheats ranging from 7% to 21% in protein content. Histogram represents the actual errors, and the full-line continuous curve the fitted normal expectation. The broken line is the fitted curve for the errors from 0.00% to 0.50% inclusive.

On the other hand, when the error distribution is normal, there is no theoretical justification for the rejection of observations on the ground of their divergence. Increased accuracy in this case must be sought solely through additional replication or improvement in experimental technique.

The above considerations naturally suggest a means of arriving at the true random error and thereby of establishing reasonable limits within which duplicates should be expected to agree. The outlying classes may be successively discarded until a normal distribution results.



The upper error limit, when this condition is attained, will then represent the expected maximum error resulting from chance variations and the point above which one is justified in discarding a result in favour of an additional single determination which agrees with one or other of the previous duplicate tests within the prescribed range.

This technique was followed and, as the extreme error ranges were successively discarded, the positive values of  $g_2$  decreased in magnitude. The wheat protein error curve was only slightly but significantly leptokurtic

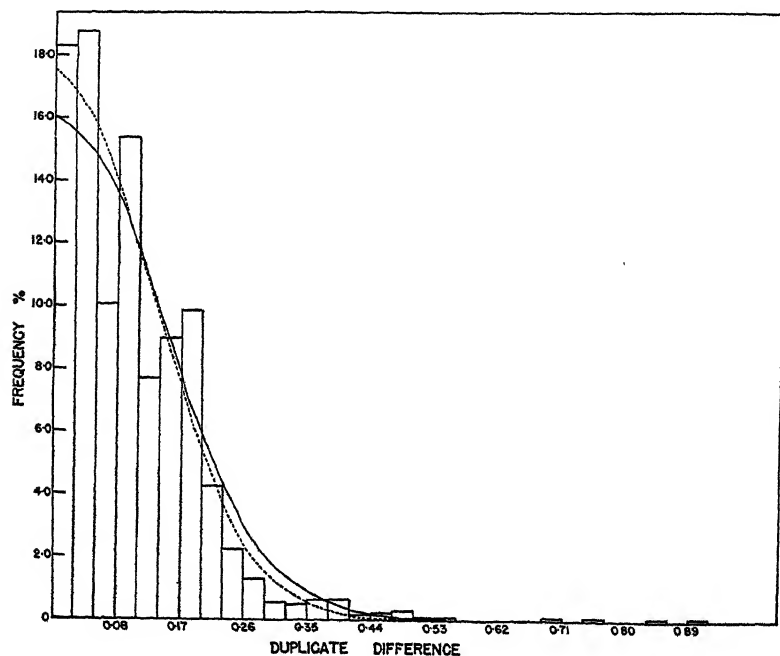


Fig. 2. Percentage frequency distribution of differences between duplicates for protein content of 1,482 flours ranging from 7% to 18% in protein content. Histogram represents the actual errors, and the full-line continuous curve the fitted normal expectation. The broken line is the fitted curve for the errors from 0.00% to 0.44% inclusive.

kurtic after all duplicate errors in excess of 0.50 were discarded; when the error class 0.48 to 0.50 was eliminated, a small but significant negative value for  $g_2$  resulted. Owing to the large number of variates involved, the test of significance is very precise and for practical purposes it may be assumed that duplicate errors within the range of 0.00 to 0.50 may be considered as ordinary experimental or random errors, whereas discrepancies in excess of 0.50 are due to accidental systematic factors. Of the 10,988 samples included in this study, 290 or 2.64% fell into this latter category.

The flour-protein-error curve for the ranges 0.00 to 0.44 inclusive was "normal"; the elimination of the class 0.42% to 0.44% rendered the curve significantly platykurtic, whereas the inclusion of the class 0.45% to 0.47% resulted in a leptokurtic distribution. Accordingly, duplicate errors up to and including 0.44% for flour protein determinations in this laboratory may be considered random and there is no justification for rejecting such observations. However, if the error exceeds this, a single additional test may be conducted and the two most closely agreeing results of the three accepted as a more reliable measure of the mean than the average of the three, provided the differences between any two of the determinations fall within the required limits. Of the 1,482 samples, 13 or 0.9% possessed duplicate errors beyond the indicated range for random errors.

The means, ranges, and standard deviations for the duplicate errors of wheat and flour for both the leptokurtic and "normal" distributions are recorded in Table III. Considering only those errors which conform

TABLE III

STATISTICAL CONSTANTS FOR ANALYTICAL ERRORS OF THE PROTEIN DETERMINATION

	No. of samples	Protein content		Differences between duplicates		Standard error (single determination)	Curve type
		Mean	Range (approx.)	Mean	Range		
Wheat	10,988	14.18	7.0-21.0	0.140	0.00-0.98	0.139	Leptokurtic
Wheat	10,698	—	7.0-21.0	0.124	0.00-0.50	0.108	Slightly leptokurtic
Flour	1,482	13.63	7.0-18.0	0.112	0.00-0.92	0.103	Leptokurtic
Flour	1,469	—	7.0-18.0	0.108	0.00-0.44	0.096	Normal

to an approximately normal distribution, the mean differences between duplicates are 0.124% for wheat and 0.108% for flour; the corresponding standard errors (single determination) are 0.108% and 0.096% respectively. It is of interest to note that Whiteside (1936), in a study of duplicate protein tests on 336 wheat samples, found the standard error of a single determination to be 0.148%. In his study, the duplicate tests were also made on the same grind and hence this figure compares with that of 0.139% found in the present study for the standard error of the entire series of wheat samples. The differences between these statistics for wheat and flour have been tested and found to be highly significant. It is of interest to note that Treloar (1930) also found the protein errors for flour to be lower than for wheat. As previously pointed out by Treloar (1929), these errors are of such magni-

tude as to render the reporting of protein results to more than one decimal place unjustifiable.

The standard errors given in Table III may be utilized to compute the number of replicates required to secure any desired degree of accuracy merely by setting up the fiducial limits at the percent point required. At the 5% point the fiducial limits will be  $\pm 1.96 s/\sqrt{n}$  where  $s$  is the standard error of a single determination and  $n$  is the number of replicates. This is based on a  $t$  value of 1.96 on the grounds that the number of samples from which  $s$  has been calculated is quite large. The results of these calculations for the flour data are given in Table IV. If only a single protein test is run, the result may

TABLE IV  
NUMBER OF REPLICATES REQUIRED FOR DIFFERENT LEVELS OF ACCURACY  
IN THE PROTEIN TEST ON FLOUR

Desired accuracy (Fiducial limits, plus or minus)	Number of replicates <sup>1</sup> ( $s = .096$ )
%	
0.20	1
0.15	2
0.10	4
0.05	14

<sup>1</sup> Given to nearest integer.

be expected to be within  $\pm 0.2\%$  of the correct value; in order to secure an accuracy of  $0.1\%$ , it would be necessary to run four replicates.

The corresponding data for wheat have not been given, because the sampling error on whole wheat was not taken into account; these might suggest that the protein content of wheat can be determined with greater accuracy than is probably the case.

### Discussion

This study, based as it is on such a large number of determinations, gives a very reliable measure of the analytical error of the Kjeldahl nitrogen test as carried out in this laboratory, and provides a practical approach for establishing standards of accuracy. The tests were made in the course of ordinary routine work and no special precautions were taken by the analysts such as is likely, consciously or unconsciously, to be the case when special studies are conducted for the purpose of evaluating errors.

It must be emphasized that the duplicate tests with wheat were made on the same grind and, hence, any error due to subsampling the grain

is not included in the statistical estimates of precision. That the error involved in sampling unground grain may frequently be a major source of error has been demonstrated by Cook, Hopkins, and Geddes (1934) in the instance of moisture determinations on wheat, and by Sallans and Anderson (1937) with respect to Lintner value determinations on malt. In view of these findings, it is altogether likely that the accuracy of the protein test, indicated in the present study, is greater than that which can be attained if the error in sampling the unground grain is taken into account. It is for this reason that the term "analytical error" rather than experimental error has been employed, since the latter properly includes errors due to sampling as well as those involved in the actual Kjeldahl determination. All samples of grain analyzed in laboratories represent some large bulk, such as a carload or the yield from a field plot, and these must be sampled at some stage of the determination. If this procedure is a serious source of error, the standard error of the protein test, if it is to be used as a criterion for determining whether two samples differ significantly, should be calculated from determinations made on duplicate subsamples ground separately. In these circumstances it appears that the principal object of making duplicate determinations on the same grind is to guard against gross errors resulting from unobserved accidents which are outside the range of normal analytical error.

It should be pointed out, however, that in many investigations, the laboratory error of the protein test, estimated from results of determinations made on duplicate samples ground separately, is not the proper one to use as a criterion of significance. A more suitable one such as a variance due to a differential effect is frequently available. This point is well illustrated in Whiteside's (1936) statistical study of protein data obtained on a series of 28 wheat varieties grown in quadruplicate row plots at each of three stations. Not only was there a significant difference between the replicate plots of each variety at each station, but there was also a significant interaction between varieties and stations, showing that the protein contents of the different varieties did not bear the same relation to each other at all three stations. Within each station, the sampling or plot error exceeded the laboratory error and, in order to secure a valid basis of comparing the varieties, it was necessary to take account of the variability in the protein content of the wheat from the different plots. Accordingly, for any one station the error variance remaining after removal of the varietal effect and that due to replications from the total variance, was the most satisfactory one to employ as a measure of the significance of differences between varieties. When, however, variety tests are conducted at a number of locations, Whiteside (1936) has shown that the interaction variance for varieties

and stations rather than the error variance should be utilized to ascertain the significance of differences between the varietal means for all stations, since the former was found to be the larger.

The problem of obtaining a good estimate of the protein content of flour is simpler than in the instance of wheat. There is no sampling error corresponding to that for unground wheat, and because flour is a very finely ground, thoroughly mixed, and relatively homogeneous material, the sampling error resulting from taking fractions for analysis should be smaller than the corresponding sampling error for ground wheat. The results of the present investigation offer some support for this hypothesis since, if the sampling error for flour is not smaller than that for ground wheat, it is difficult to understand why the standard error for the determination of flour protein is significantly lower than that for wheat protein.

It is of interest to note that Treloar (1932), in a study of the protein errors of quadruplicate determinations, reported by 99 laboratories on a single flour sample, found that while the composite error curve was symmetrical, the errors in the extreme ranges appeared to be of greater frequency than the "normal" distribution would allow for. However, the distribution of error for individual laboratories may be normal but if the standard deviations of these errors be differentiated the composite curve of the errors of all collaborators combined would be leptokurtic. A study of the distribution of the standard deviations of error for the various laboratories indicated that such differentiation existed and could easily be responsible for the leptokurtic character of the composite error curve. Treloar (1932) points out that these observations cannot be interpreted as implying that the error distribution within laboratories is normal; he considers that the hypothesis of normality of error distribution provides a satisfactory basis of deduction for practical purposes, since any error it introduces would be small.

The data presented in this paper show quite conclusively that the protein-error curve is leptokurtic. Reference has been made to the fact that loaf-volume errors have also exhibited this type of abnormality. Upon reflection, it would be anticipated that in any laboratory where large numbers of routine determinations are being carried out daily, unobserved accidental discrepancies are bound to occur, which result in occasional errors of larger magnitude than the normal range. In general, then, leptokurtic distributions would seem to be the rule rather than the exception and this is a matter of great importance in establishing standards of error for such analytical procedures. With such distributions, the rejection of outlying observations is perfectly justified and may be expected to result in enhanced precision. A large number

of observations, however, are necessary in order to fix the range within which errors may be considered due to random causes.

### Summary

A study of the analytical errors of duplicate protein tests on 10,988 wheats varying between 7% and 21% in protein content and on 1,482 samples of flour containing from 7% to 18% in protein reveals that no relation exists between protein content and the magnitude of the errors.

The analytical errors ranged from 0.00% to 0.98% for wheat and from 0.00% to 0.92% for flour; the corresponding mean errors were 0.140% and 0.112%, while the standard errors of a single determination were 0.139% and 0.103% respectively. As the duplicate tests with wheat were made on the one grind, any errors involved in sampling the whole grain are not included.

The error distributions were leptokurtic, indicating the occurrence of gross discrepancies, probably resulting from unnoticed minor accidents which are outside the range of normal analytical error. Successive rejection of outlying observations resulted in a normal error-distribution curve over the range of 0.00% to 0.50% for wheat and 0.00% to 0.44% for flour. These ranges represent the limits within which duplicate errors may deviate from random causes alone, and results which deviate beyond these limits may be discarded.

The random protein error for wheat is significantly higher than for flour; this is most probably the result of a greater sampling error due to the less homogeneous and less finely ground state of the former. The mean random errors are: for wheat 0.124% and for flour 0.108%; the corresponding standard errors of single determinations are 0.108% and 0.096% respectively. Assuming a negligible sampling error for flour, single tests may deviate from the true value by 0.2%, and in order to secure a protein result accurate to 0.1%, four replicates agreeing within the specified ranges for random error would be necessary.

### Acknowledgments

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## A STUDY BY THE PAIRED FEEDING METHOD OF THE NUTRITIVE VALUE OF BREAD MADE WITH MILK SOLIDS<sup>1</sup>

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In 1937, this laboratory undertook the study of the comparative nutritive value of three types of dry bread crumb prepared from bread doughs containing 0%, 6% and 12% of milk solids based upon weight of flour. A preliminary report has been published (Fairbanks, 1938) in which the *ad libitum* method of feeding was employed and the conclusions drawn were based entirely upon differences in body weight. The plan of work was stated as follows: "It is the purpose of this present work to compare the over-all nutritive value of bread made without milk solids, with bread made with the addition of 6% milk solids (based upon weight of flour) and with bread made with the addition of 12% milk solids. The experimental technique employed in this initial work is not capable of explaining in terms of nutrition why observed differences occur. At present we are only interested in the differences, and further experiments which are better controlled and more refined in procedures will be conducted to explain that which has been observed and recorded here."

One must carefully avoid very emphatically expressed judgments based upon differences in body weights as obtained from *ad libitum*

<sup>1</sup> This investigation was made possible by the donation of funds to the University of Illinois by the American Dry Milk Institute, Inc.

feeding. Such differences may be due merely to differences in palatability rather than to differences in nutritive efficiency. The effects of palatability may be ruled out by modern methods of controlled feeding. Differences in body weights may also fail as an index to nutritive value, as they do not account for possible differences in body composition. This objection is readily overcome by making carcass analyses at the close of the feeding experiment.

This paper reports the results of an experiment in which the controlled-feeding technique was employed and the carcasses of the experimental animals were subjected to chemical analysis at the close of the feeding period. It is hoped that by this more critical experimental approach we may be able to confirm the conclusion previously reported (Fairbanks, 1938) that "The addition of milk solids to a water bread (no milk) formula increases the nutritive value of bread."

The bread samples used were the same as those studied in the preliminary experiment and, to conserve space, the reader is referred to the previous paper for a description of the samples, formula used, methods of baking, and preparation of the samples following baking. Bread samples were prepared at two different times and the chemical composition of both makes are presented in Table I. The chemical

TABLE I  
CHEMICAL COMPOSITION OF THE EXPERIMENT NO-MILK-SOLIDS BREAD  
AND 12 PERCENT MILK-SOLIDS BREAD

Sample	Dry sub- stance	Ether extract	Crude protein (N X 6.25)	Gross energy per g.	Ash	Cal- cium	Phos- phorus
	%	%	%	cal.s.	%	%	%
No-milk bread, 1st make	96.08	0.98	15.88	4248	2.33	0.026	0.141
6% milk-solids bread, 1st make	96.27	0.86	17.01	4241	2.74	0.097	0.187
12% milk-solids bread, 1st make	96.41	0.90	17.68	4219	3.00	0.162	0.236
No-milk bread, 2nd make	95.22	0.95	13.08	4181	2.28	0.033	0.118
6% milk-solids bread, 2nd make	95.11	0.92	14.42	4160	2.63	0.108	0.180
12% milk-solids bread, 2nd make	95.66	0.85	15.76	4171	2.97	0.178	0.227

analyses indicate that the greatest differences occur in the protein, ash, calcium, and phosphorus contents of the no-milk, 6%, and 12% milk-solids bread.

The literature was reviewed and included in the former publication.

### Plan of Experiment

The experimental technique employed was the paired-feeding method (Mitchell and Beadles, 1930) slightly modified to include three rats in each group rather than the customary two rats. Seven groups



of three albino rats each were carefully equated on the basis of litter, sex, and body weight. The first rat of each group was fed no-milk bread, the second rat received 6% milk-solids bread, and the third rat was fed 12% milk-solids bread. The sole diet of these experimental animals was bread and distilled water. This severe dietary regime coupled with the controlled method of feeding must be kept in mind as one studies the data.

The rats were confined in individual wire cages, and the food was weighed out daily to each individual rat. Food scattering was practically eliminated by mixing distilled water into the bread crumb after weighing. At the close of the experiment the rats were sacrificed, their body lengths taken immediately after death, and their carcasses saved for chemical analyses.

In the controlled feeding technique there are two methods that may be used. The first method prescribes the feeding of the rats of any one group of three rats so that the gains in body weight will be equal. This method rules out differences in maintenance requirements due to differences in body weights. The nutritive differences of the diets are indicated by differences in body lengths and food consumption for equal gains. The second method of controlled feeding is accomplished by feeding the same amount of feed to all rats of any one group. Differences in body lengths and differences in body weights may be regarded as measures of nutritive value. The carcass analyses contribute to the data obtained by either method of feeding.

When the experiment was initiated, it was planned to feed for equal gains. Because of the very poor performance of two groups of rats, it was deemed advisable at the end of 49 days to change over to the method of feeding equal amounts to the three rats of each group. When this change was made the rats were put under ether and their body lengths were taken. Thus for the first 49 days, data on food intake, body weights, and body lengths are available. After the change was made to equalized food intake, the experiment was continued for 56 days. Table II, which is divided into two parts, gives the essential data. The first part is for the 49 days on feeding for equal gains, the second part for the 56 days on equalized food intake.

If a rat of any one group died during the experiment, its group mates were sacrificed on the same day, and the three carcasses saved for chemical analysis. At the close of the experiment, three groups of rats had survived the rigorous experimental conditions and they were disposed of and their carcasses saved. Table III presents the chemical analysis of the seven groups consisting of 21 rats. All analyses are calculated on the dry-matter basis.

TABLE II  
BODY LENGTHS AND BODY WEIGHTS, GAINS IN BODY WEIGHT AND FOOD CONSUMPTION ON THE THREE SAMPLES OF BREAD CRUMB  
(All weights expressed in grams)

	Group 1			Group 2			Group 3			Group 4			Group 5			Group 6			Group 7		
	No milk		6 %	No milk		6 %	No milk		6 %	No milk		6 %	No milk		6 %	No milk		6 %	No milk		6 %
	1f	2f	3f	4m	5m	6m	7f	8f	9f	10m	11m	12m	13f	14f	15f	16f	17f	18f	19m	20m	21m
Rat no. and sex																					
Initial body weight	47	53	56	54	57	53	53	50	51	50	52	62	51	52	50	51	58	50	54	56	58
Final body weight	57	65	66	86	91	81	59	65	59	63	68	76	63	54	54	51	49	51	99	96	97
Gain	10	12	10	32	34	28	6	6	8	13	16	14	12	2	4	0	1	1	45	40	39
Body length, mm. <sup>1</sup>	144	155	158	163	174	187	151	153	153	157	167	154	154	163	163	148	149	158	168	172	177
Food	188	183	168	245	239	222	200	179	194	226	195	220	213	211	193	194	211	210	350	283	302
Length of test in days	49	49	49	49	49	49	49	49	49	49	49	49	44	44	44	49	49	49	49	49	49
FEEDING FOR EQUAL GAINS																					
EQUALIZED FOOD INTAKE																					
Initial body weight	—	—	—	86	91	81	59	56	59	63	68	76	—	—	—	51	49	51	99	96	97
Final body weight	—	—	—	98	119	118	75	85	88	68	86	84	—	—	—	53	53	60	77	97	104
Gain	—	—	—	12	28	37	16	29	29	5	18	8	—	—	—	2	4	9	-22	1	7
Body length, mm. <sup>1</sup>	—	—	—	165	176	189	157	166	167	160	172	177	—	—	—	152	157	161	168	182	188
Food	—	—	—	283	283	283	217	217	217	238	238	238	—	—	—	187	187	187	191	191	191
Length of test in days	—	—	—	49	49	49	56	56	56	56	56	56	—	—	—	56	56	56	32	32	32

<sup>1</sup> Measured from anus to tip of nose.

TABLE III

CHEMICAL COMPOSITION OF THE CARCASSES OF THE RATS RECEIVING NO-MILK BREAD, 6 PERCENT MILK-SOLIDS BREAD AND 12 PERCENT MILK-SOLIDS BREAD  
(Results calculated on a dry-matter basis)

Bread	Rat no. and sex	Dry substance	Gross energy per gram	Protein (N×6.25)	Ether extract	Ash	Calcium	Phosphorus
No-milk bread	1f	30.68	4955	67.03	14.64	9.71	2.47	1.93
6% milk bread	2f	29.43	5433	73.10	12.51	12.34	3.25	2.37
12% milk bread	3f	31.89	5115	75.77	8.35	13.60	3.56	2.60
No-milk bread	4m	34.10	5256	68.50	23.15	7.88	1.86	1.56
6% milk bread	5m	32.71	6362	64.44	34.85	9.70	2.54	1.77
12% milk bread	6m	34.89	5209	57.92	23.39	10.07	3.14	1.97
No-milk bread	7f	35.62	6833	60.95	35.04	8.91	2.29	1.71
6% milk bread	8f	34.49	5936	57.10	30.57	9.92	2.75	2.03
12% milk bread	9f	33.06	6405	56.81	33.79	11.72	3.50	2.26
No-milk bread	10m	32.37	5043	72.03	13.16	9.73	2.16	1.93
6% milk bread	11m	26.28	5787	78.83	17.26	13.91	4.03	2.72
12% milk bread	12m	30.18	4687	76.33	8.94	14.53	4.13	2.74
No-milk bread	13f	28.55	5212	79.84	10.01	11.48	2.96	2.24
6% milk bread	14f	35.04	3862	71.62	4.30	12.20	3.41	2.32
12% milk bread <sup>1</sup>	15f	—	—	—	—	—	—	—
No-milk bread	16f	34.36	5229	58.03	29.04	12.63	2.79	2.02
6% milk bread	17f	30.33	5828	67.02	18.68	14.97	4.50	2.85
12% milk bread	18f	35.10	4928	60.22	16.10	13.65	4.67	2.57
No-milk bread	19m	27.30	4429	88.54	4.58	11.16	2.90	2.13
6% milk bread	20m	32.50	5122	77.18	10.26	11.58	3.21	2.18
12% milk bread	21m	31.42	5063	79.69	11.61	13.13	3.67	2.68
Averages								
No-milk bread	—	—	5280	70.70	18.52	10.21	2.49	1.93
6% milk bread	—	—	5476	69.90	18.35	12.09	3.38	2.32
12% milk bread	—	—	5235	67.79	17.03	12.78	3.78	2.47

<sup>1</sup> Sample lost.

The data have been subjected to statistical analysis according to the method of Student (1908) and the statistical results are summarized in Table IV.

### Results of the Experiment

*Feeding for equal gains.*—In the first part of Table II will be found the data for the 49 days of feeding for equal gains. It will be noted that we were not entirely successful in keeping the gains of all three rats in each group exactly the same. As the experiment progressed this became increasingly difficult, because of erratic appetites of the rats, especially those receiving the no-milk bread. For this reason, along with poor performance of Groups 5 and 6, the change in feeding was

made as has been previously mentioned. However, the data on body lengths and on food consumption may be used as indices of differences in the nutritive values of the three kinds of bread crumb.

The average body length of the rats receiving no-milk bread was 154 mm., for the rats fed 6% milk-solids bread, 160 mm., and for the rats on 12% milk-solids bread, 166 mm. If the differences obtained with the rats on the three kinds of bread are considered as paired observations, then Student's (1908) method for statistical analysis of small groups of such data may be applied. As seen in Table IV, the average difference in body length between the rats fed no-milk bread and 6% milk-solids bread is 6.00 mm., the standard deviation is 3.93, and the probability of a chance outcome only 0.0048. When the body lengths of the rats on no-milk bread are compared with those fed 12% milk-solids bread, the average difference is 11.71 mm., the standard deviation of excess length is 6.26, and the probability that fortuitous factors would have produced this outcome is only 0.0019. The figures for the differences in body length between 6% milk-solids bread and 12% milk-solids bread are 5.71 mm. for the difference, with a standard deviation of 4.72 and a probability figure of 0.0127. In all three instances the criterion of significance has been satisfied. Such statistical evidence supports the conclusion that the rats on the 6% and 12% milk-solids breads are significantly longer than the rats fed the no-milk bread, and that the rats on the 12% milk-solids bread are significantly longer than those fed 6% milk-solids bread. This observation is emphasized as there is a growing conviction in this laboratory that differences in body lengths are a more superior criterion of nutritive efficiency in the growing rat than differences in body weights.

The average food consumption of the rats receiving no-milk bread was 231 g., for those receiving 6% milk-solids bread it was 214 g., while the average consumption of the rats on 12% milk-solids bread was 216 g. As indicated in Table IV these differences in food consumption are not statistically significant. In other words significant differences in the body lengths of the three series of rats have been obtained on food intakes, the differences between which are not statistically significant, an observation that may be used as further evidence of the nutritional superiority of the breads containing the milk solids. In this part of the experiment the rats were being fed for equal gains, so greater nutritive efficiency would be indicated by a smaller food intake. It is admitted that a stronger case for the addition of milk solids could be presented if the food consumption of the rats receiving milk-solids bread decreased as the milk solids were increased, with observed differences that were statistically significant. Referring again to the statistical analyses in Table IV, it is interesting

TABLE IV

ANALYSIS ACCORDING TO STUDENT'S METHOD OF THE OBSERVED DIFFERENCES WITH  
RATS FED NO-MILK BREAD, 6 PERCENT MILK-SOLIDS BREAD  
AND 12 PERCENT MILK-SOLIDS BREAD

	Mean of difference m	Standard deviation of difference s	Probability p	Statistical significance <sup>1</sup>
<b>Feeding for equal gains:</b>				
Body lengths				
No milk vs. 6%	6.00	3.93	0.0048	S
No milk vs. 12%	11.71	6.26	0.0019	S
6% vs. 12%	5.71	4.72	0.0127	S
Food consumption				
No milk vs. 6%	16.43	24.94	0.0816	N
No milk vs. 12%	15.29	18.22	0.0432	N
6% vs. 12%	1.14	17.08	>0.4072	N
<b>Equalized food intake:</b>				
Gains				
No milk vs. 6%	13.40	6.77	0.0084	S
No milk vs. 12%	15.40	10.07	0.0189	S
6% vs. 12%	2.00	6.66	0.2909	N
Body lengths				
No milk vs. 6%	10.20	3.06	<0.0019	S
No milk vs. 12%	16.00	5.76	0.0026	S
6% vs. 12%	5.80	3.97	0.0218	S
<b>Carcass analysis:</b>				
Protein				
No milk vs. 6%	0.80	7.44	0.3985	N
No milk vs. 12%	1.39	7.02	0.3366	N
6% vs. 12%	1.82	3.84	0.1718	N
Ether extract				
No milk vs. 6%	0.17	7.07	0.4072	N
No milk vs. 12%	2.91	6.12	0.1670	N
6% vs. 12%	3.66	5.10	0.0846	N
Energy				
No milk vs. 6%	196.14	862.07	0.2977	N
No milk vs. 12%	56.33	367.71	0.3762	N
6% vs. 12%	510.17	593.34	0.0568	N
Ash				
No milk vs. 6%	1.88	1.22	0.0047	S
No milk vs. 12%	2.78	1.25	0.0021	S
6% vs. 12%	0.71	1.04	0.0950	N
Calcium				
No milk vs. 6%	0.89	0.59	0.0051	S
No milk vs. 12%	1.37	0.42	<0.0006	S
6% vs. 12%	0.40	0.23	0.0058	S
Phosphorus				
No milk vs. 6%	0.39	0.29	0.0085	S
No milk vs. 12%	0.59	0.12	<0.0006	S
6% vs. 12%	0.15	0.24	0.1098	N

<sup>1</sup> S = significant, N = not significant.

to note that this situation was approached in the comparison between no-milk and 6% milk-solids bread and again between no-milk bread and 12% milk-solids bread. While the differences are not statistically significant and the data are therefore not conclusive, the probability values do suggest that the 6% milk-solids bread and the 12% milk-solids bread when compared to the no-milk bread have produced equal gains on less food intake. This is particularly true in the comparison of no-milk bread with 6% milk-solids bread, as the probability figure is 0.0432. It must be remembered that the numbers of paired observations are small and the experimental conditions imposed are quite severe, making for erratic appetites.

The observations during the period of feeding for equal gains substantiates the conclusion that both the 6% and 12% milk-solids bread are more nutritious than the no-milk bread and that the 12% milk-solids bread has a higher nutritive value than the 6% milk-solids bread.

*Equalized food intake.*—The data during the period of feeding the same amount of food to the rats of any one group are presented in the second part of Table II. In this phase of the experiment, differences in nutritional efficiency are demonstrated by differences in gains in body weight and differences in body lengths.

The average gain in body weight for the rats fed no-milk bread was 2.6 g., on 6% milk-solids bread, 16 g., and on 12% milk-solids bread, 18 g. Further evidence of the improvement in nutritive value of bread by the addition of milk solids is afforded by the significant differences of the gains between no-milk bread and 6% milk-solids bread, as the probability of a chance outcome is 0.0084 (Table IV). The gains between the no-milk bread and the 12% milk-solids bread is likewise statistically significant with a probability of 0.0189. However, in this particular measurement we are unable to show a significant difference between gains in body weight of rats fed 6% and 12% milk-solids bread. As seen in Table IV the mean of the difference is only 2 g. in favor of the 12% milk-solids bread, while the standard deviation of the difference is 6.66 with a probability of 0.2909, which is definitely not significant.

At the close of the second phase of the experiment, body lengths were again measured and are included in Table II. The statistical analyses of these data are presented in Table IV. The rats receiving 6% milk-solids bread and 12% milk-solids bread were significantly longer than those receiving no-milk bread. Further, the rats fed 12% milk-solids bread were significantly longer than those on 6% milk-solids bread. It must be pointed out that while these differences in body lengths are due to differences in the nutritive values of the three

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## STUDIES ON ALL-PURPOSE FLOUR

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Although much work has been done during recent years on the chemical properties and baking qualities of various flours, relatively little attention appears to have been paid to all-purpose flour, that widely selling variety most commonly used by housewives. This is in spite of the fact that the number of flours which bear the name "all-purpose" is legion. Flours from all over the country, and in a widely varying price range, are labeled in this way. When it is considered that these flours represent not only different varieties of wheat but also differences in consumer demand, it seems not unlikely that there might

be necessity for some elasticity in the use of the term. In view of these considerations a study of the baking qualities of several all-purpose flours from different wheat-growing and milling centers seemed desirable.

Five flours were chosen for the study. One was a Texas product, one was from Indiana, another was milled in Kansas City, while the remaining two were milled in Minnesota but were of different protein contents.

These flours were analyzed for total nitrogen and moisture content by the official methods of the Association of Official Agricultural Chemists. They were also used to make butter cake, yeast bread, and baking-powder biscuits, all products widely prepared by housewives. The products, which were baked by standard recipes under controlled conditions, were judged by both subjective and objective methods.

Table I shows the results of the chemical determinations and the averages of the judges' scores on three series of each product. The highest possible score in each case was 100.

TABLE I  
CHEMICAL RESULTS AND JUDGES' SCORES FOR FIVE ALL-PURPOSE FLOURS

Flour	Protein content	Moisture content	Score on cakes	Score on bread	Score on biscuits	Total score
	%	%				
Texas brand	10.91	12.03	91.2	87.6	86.3	265
Indiana brand	10.54	12.71	87.5	86.2	85.9	259
Minnesota brand 1	10.98	12.43	90.1	87.9	83.3	260
Minnesota brand 2	11.70	13.01	90.7	89.3	88.6	269
Kansas City brand	10.89	11.85	90.2	85.4	88.1	263

It will be seen that the flour with the highest protein content was marked by the judges as making the best loaf of bread. This is as might be expected, since it has been shown that there tends to be a relationship between baking quality and protein quantity. Flours with a high protein content appear to be best for bread, while lower-protein flours make better cakes. In this study, however, the flour with the lowest protein percentage had the lowest rating on cakes.

The figures in the final column of Table I show that scores for all of the flours do not vary widely, indicating that products of a fairly similar nature were obtained. As a general rule, a flour which scored high on one product usually scored high on the others, too.

Several objective tests were tried on the baked products. For the cakes the index to volume and the compressibility were determined



according to methods described by Platt (1930) and Platt and Kratz (1933), and the moisture and sand-retention tests of Swartz (1938) were also carried out. In the experimental work with bread, the index to volume, compressibility, oven spring, and amount of water required to make a dough were determined. Biscuits were rated only on the basis of judges' scores. The various tests are described below.

By use of a planimeter, the surface area of the center section of each cake was determined. In making the measurements, the cakes were cut through the center, the exposed surfaces were placed against a piece of paper, and the outline traced in pencil. Two readings, checking within 0.02 square inch, were taken for each half. The results were averaged and reported as the surface area. There is a relation between surface area and volume, and it is possible to calculate the volume of the cake in cubic centimeters from this value. In this study, however, the method of Platt and Kratz (1933) was followed, and the planimeter readings themselves are reported as the "index to volume." This index for cakes made from each of the all-purpose flours varied only slightly. It might be observed, however, that the cakes which the judges scored highest had a slightly higher volume, while those with the lowest score also had the smallest volume.

The compressibility of cakes made from each flour was determined by use of the compressometer, a device developed by Platt (1930). By this method a weight is allowed to rest on the cut surface of the cake for one minute. It was observed that high compressibility was not necessarily an indication of quality for all-purpose flour cakes. Rather, it appeared that a cake having a coarse grain offered less resistance to the plunger of the compressometer than those with a fine grain and smooth texture.

The moisture absorption test is, to a certain extent, a measure of the eating qualities of a cake. Swartz (1938) suggests that probably the dry, unpleasant feel of a poor cake in the mouth is due to the fact that it does not absorb the saliva as rapidly as a more moist one. In performing the test, samples of cake are cut, weighed, placed in a petri-dish cover containing 30 cc. of water, and allowed to remain for exactly five seconds. At the end of that time the sample is removed, inverted, and quickly reweighed. The difference in grams between the two weights is taken as a measure of the absorptive power of the cake. The test may also be used as a measure of keeping qualities, since the cakes tend to absorb less moisture as they become drier and older. Little difference could be seen in moisture-absorption powers between the cakes made from the different flours, the average increase in weight being 11.30 grams.

The sand-retention test is designed as a relative measure of the size of grain of cakes. The test is made by cutting a sample of cake of definite size, weighing it, covering it liberally with sand of a known fineness, removing the excess sand by rotating the sample once at a 40-degree angle, and weighing again. A cake having a coarse, open grain should retain more sand than a fine-textured one. Here again no significant difference could be seen between cakes made from different all-purpose flours.

It should be stated that while cakes made from each of the all-purpose flours were good on the whole, none of them had as velvety a texture nor as even a grain as cake-flour cakes made under the same conditions. One series of cake-flour cakes was made during the course of the experiment. It was observed that while all-purpose-flour cakes have a smaller volume, are less compressible, and have a smaller moisture-absorption power than cakes made from cake flour, they appear to have better keeping qualities.

Table II shows the results of the work on cakes. The figures represent the averages of determinations on nine cakes from each all-purpose flour, and three cake-flour cakes.

TABLE II  
RESULTS OF OBJECTIVE TESTS ON CAKES

Flour	Index to volume	Compressibility <sup>1</sup>			Moisture absorption <sup>1</sup>			Sand retention <sup>1</sup>
		1st day	2nd day	4th day	Fresh sample	After 3 days	After 1 wk.	
	<i>sq.in.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>g.</i>	<i>g.</i>	<i>g.</i>	<i>g.</i>
Texas brand	9.75	24.8	17.7	13.1	11.83	11.18	7.98	1.61
Indiana brand	9.38	25.9	19.0	13.9	11.62	10.30	8.70	1.65
-Minnesota brand 2	9.67	23.3	17.9	14.2	11.24	9.13	8.67	1.69
Minnesota brand 1	9.67	23.9	19.1	15.1	10.49	10.35	5.79	1.45
Kansas City brand	9.62	23.1	15.6	13.5	11.36	8.51	8.08	1.61
Cake flour	11.61	32.2	22.7	14.9	14.28	8.70	—	1.62

<sup>1</sup> Determinations made on samples 2 inches in diameter and 1 inch thick.

The index to volume of the breads was measured in the same way as that of the cakes. The volume of loaves made from Minnesota flour 2, that having the highest protein content, was found to be the largest, while those made from the Indiana flour, which had the smallest protein content, had the smallest volume.

When the compressibility of the breads was measured, four of the flours showed similar results, with an average compressibility on the first test of 11.9 mm. Bread made from Minnesota brand 1, however,

showed considerably greater compressibility than the other breads, its compressibility on the first test averaging 16.2 mm. The reason for this is not clear.

No significant difference could be seen between the flours in oven spring, or in the amount of water required to make a dough.

The results of the objective tests on bread are summarized in Table III.

TABLE III  
RESULTS OF OBJECTIVE TESTS ON BREAD

Flour	Volume	Compressibility		
		1st day	2nd day	4th day
	<i>sq.in.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Texas brand	14.07	11.2	7.9	6.1
Minnesota brand 2	14.56	12.3	8.1	6.2
Minnesota brand 1	14.18	16.2	11.1	8.9
Kansas City brand	13.60	13.3	9.3	6.6
Indiana brand	13.17	10.9	8.7	6.6

In the case of biscuits, it was found that on the main points of texture, flavor, crumb color, and volume, the flours ranked high as a whole. There was considerable variation on the points of symmetry, crust color, and character of crust.

It appears that although the five flours came from different parts of the country, had different protein contents and doubtless represented a number of varieties of wheat, the products made from them showed relatively little variation. It is to be remembered that flours may vary greatly in their baking qualities with changes in recipe or in methods of mixing. The standard conditions set up in this experiment were not necessarily the ideal ones for all of the flours. The results were similar enough so that it was felt that with careful though not necessarily similar handling, good products could be obtained from all of the flours. On the basis of these conclusions, therefore, it appears that all of the flours tested may rightfully bear the label "all-purpose."

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# OBSERVATIONS ON THE HYDROGEN-ION CONCENTRATION OF CAKES<sup>1</sup>

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The optimum hydrogen-ion concentration of various types of cakes for best flavor and appearance has not been definitely known. While the effects of increased acidity or alkalinity on the cake volume, grain, and texture can be determined quite accurately, the measurement of flavor and eating quality is difficult because of different individual preferences. Observations on the pH of white and yellow layer cakes and the effect of certain materials on the pH of some cakes will be presented here.

In one series of tests a yellow layer cake was baked after the following basic formula: 100% cake flour, 50% shortening (hydrogenated), 125% sugar (2X), 50% whole eggs (frozen), 3% salt, 7% baking powder (phosphate), 15% dry milk solids,<sup>3</sup> and 87% water and flavor. All mixing and baking procedures were maintained constant. The percentages of the various ingredients were varied and the resulting changes in pH of the cake were observed. The pH was obtained by shaking 10 g. of cake crumb in 50 c.c. of distilled water and allowing this to stand for 30 minutes with thorough shaking at intervals. The suspension was then centrifuged and the supernatant liquid was immediately used for pH determinations by means of a potentiometer. Garnatz (1937) showed that extracts of baked products will decrease in pH upon standing, so delays were avoided in the final stages of the determination.

The results of the tests indicated that variations within limits in the amount of shortening, sugar, whole eggs, and water did not affect the pH of the cakes appreciably. Baking powder was used at 6.5, 7.0, and 7.5% levels and the cakes had pH values of 7.35, 7.74, and 7.91, respectively. Thus a variation in the amount of baking powder in the batter produces a considerable change in pH of the cake. Dry milk solids used at levels of 8, 15, 20, 25, and 30% and with variations in the amount of water to maintain a constant viscosity of the batter resulted in pH values of 7.70, 7.41, 7.56, 7.31, and 7.29, respectively. This indicates a slight trend of the pH toward neutrality with increasing amounts of milk solids in the instance of this particular cake formula.

<sup>1</sup> Paper No. 1660, Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

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<sup>3</sup> Dry milk solids is the product resulting from the removal of fat and water from milk. It contains not over 1½% butterfat and not over 5% moisture.

Similar studies were also made on white layer cakes and the results were practically the same as those described for the yellow layer cakes.

One series of yellow and one of white layer-cake batters were then made, to which various increments of sodium bicarbonate or potassium acid tartrate were added in addition to the regular amount of phosphate baking powder. These cakes were scored for color of crust, symmetry, volume, texture, grain, crumb color, and eating quality. The same yellow-cake formula was used as before. The white-cake

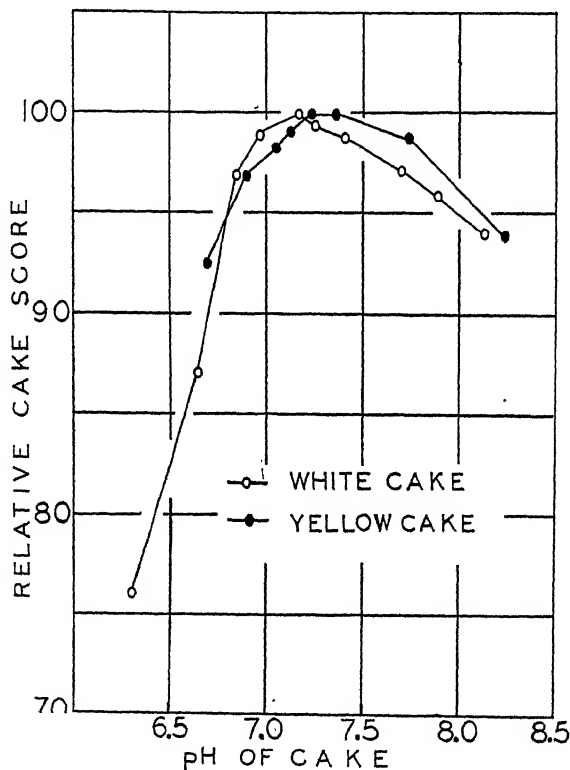


Fig. 1. Showing the effect of pH of the cakes on the final scores.

basic formula was as follows: 100% cake flour, 110% sugar, 52.5% shortening, 50% egg white (frozen), 30% dry milk solids, 7% baking powder, 3.75% salt, and 90% flavor and water. The flavoring substance used in all tests was a very small amount of pure vanilla extract. The scores obtained for the cakes on the basis of 100 for the best cake are shown in relation to the pH of the cakes by the graph in Figure 1. The highest scores were on the slightly alkaline side with both types of cakes and within a pH range from 7.00 to 7.90. The cakes with a pH

in this range were superior in all respects to the cakes which were either more acid or more alkaline.

White cakes at pH 8.15 had a dark crust and a yellowish crumb and this was also noticed to some extent at pH 7.88. The yellowish crumb color produced in white cakes by high alkalinity is probably due to a change in color of some of the flour pigments and also to greater caramelization of the sugars at the higher pH, particularly in the crust. When the acidity was increased beyond neutrality (pH 7.0) the crust color became appreciably lighter, in both the yellow and the white cakes.

To obtain some additional opinions relating to flavor, a group of ten persons were asked to judge the cakes as to preference. The yellow cakes at pH 7.19 and 7.25 were generally preferred, although a pH range of 6.98 to 7.88 was also acceptable to some persons. The acceptable white cakes were in the range from pH 7.05 to 7.89, but the cakes at pH 7.22 and 7.35 were commonly regarded as superior. Judges who were asked to taste cakes which were a day old did not as a rule exactly duplicate their opinions of the fresh cakes, but their selections were within the pH range of 7.10 to 7.80.

The series of tests just described were duplicated except that a commercial tartrate baking powder was used instead of the phosphate baking powder, with practically the same results. It was observed, however, that from the batters with no added soda or cream of tartar the tartrate-baking-powder cakes were at pH 7.13 and 6.90 and the phosphate-baking-powder cakes at pH 7.74 and 7.71 for the yellow and the white cakes, respectively. Thus the phosphate powder was balanced to give a more alkaline reaction than the tartrate powder that was used.

To study the effect of aging upon pH, white cakes made with tartrate baking powder were stored in a cabinet for three days at room temperature. The resulting data are shown in Table I. There was

TABLE I  
EFFECT ON PH OF WHITE LAYER CAKES UPON THREE DAYS'  
STORAGE AT ROOM TEMPERATURE

Fresh cakes	8.06	7.96	7.67	7.50	7.15	6.90	6.83	6.78	6.67
Three days old	7.90	7.86	7.57	7.47	7.10	6.93	6.88	6.88	6.76

only a slight change in pH during the three days of storage. The small change appears to be toward neutrality of both the acid and the alkaline cakes. This is in agreement with the observations of Karacsonyi (1928) on the change in pH of bread stored at room temperature for 48 hours. He concluded that the acidity either remained constant or showed some decrease upon storage.

Several white, yellow, chocolate, and angel food cakes from various commercial bakeries were obtained and the pH was determined. The values are shown in Table II. The average pH for white cakes was

TABLE II  
THE PH OF SOME COMMERCIAL CAKES FROM VARIOUS TYPES OF BAKERIES

Type of bakery	White cakes	Yellow cakes	Chocolate cakes	Angel food cakes
Chain retail	7.08	—	7.96	5.33
Chain retail	7.29	—	7.93	—
Chain retail	7.68	—	8.63	—
Retail	7.22	7.68	8.89	6.48
Retail	7.95	7.69	8.81	—
Retail	—	—	8.47	—
Wholesale	7.61	7.39	8.49	5.44
Wholesale	—	—	8.71	5.43
Av. pH	7.47	7.59	8.48	5.67

7.47, yellow cakes 7.59, chocolate cakes 8.48, and angel food cakes 5.67. Most of the commercial white and yellow cakes were within the optimum pH range as previously determined by this study.

Glabau (1938) placed the optimum pH for white and yellow cakes at about pH 7.00, but it should be noted that the pH differences between the individual cakes in his studies were rather large. Accordingly it became difficult to narrow down the optimum range within the smaller limits, which was attempted in this study. He pointed out that pH control of cakes is important and that cakes with egg yolks and no whites will have a higher acidity than cakes with whole eggs, and these in turn higher than with egg white when all other factors are constant.

Cake ingredients such as baking powder, eggs, flour, and liquid milk from different sources vary appreciably in their buffering action in the cake batter. Six lots of spray-process dry milk solids were available and yellow cakes baked with 30% milk solids had pH values of 7.12, 7.34, 7.27, 7.25, 7.41, and 7.27, respectively. In these instances all cakes scored high and were of good quality, and apparently these different high-grade milk solids varied only slightly in their effect on the pH of the finished cake.

### Summary

Baking powder, in terms of both kind and quantity, affected the pH of white and yellow layer cakes more than any of the other common ingredients.

Optimum pH range of white and yellow layer cakes, in terms of preferred flavor and eating qualities, was 7.0–7.9. It appears that this

range could be narrowed down to 7.22-7.35. Cakes with a pH value outside the range of 7.0-7.9 were distinctly inferior when baked with either a phosphate or a tartrate baking powder.

Dry milk solids affected the pH of cake to a limited and probably an insignificant degree when used in proportions of 8 to 30 parts per 100 parts of flour in the cake formula. Likewise, there was only a small difference in the pH of cakes made with six different commercial lots of high-grade dry milk solids.

Storing cakes for three days did not result in an appreciable change in pH.

Average pH value of commercial white cakes was 7.47; of commercial yellow cakes 7.59; of chocolate cakes 8.48; and of angel food cakes 5.67.

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## "PHOTO-RECORDS" AS APPLIED TO CAKE

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In a previous article a method and apparatus were described for making permanent records of baking studies.<sup>1</sup> Most of the work reported in this first article dealt with bread. One illustration of pound cake was shown and it was only fairly satisfactory. All pictures of cake obtained up to that time were more or less "fogged" around the edges because of stray light. This article presents some additional work in the production of "photo-records" of cakes.

### Apparatus

All of the pictures recorded here were made with the "photo-record" apparatus<sup>2</sup> which was described briefly in the supplement of the pre-

<sup>1</sup> These projected photographic pictures will be referred to as "photo-records." The previous article is Wm. H. Cathcart, A Practical Method of Obtaining Permanent Records of Baking Studies, *Cereal Chem.* 15: 775-787.

<sup>2</sup> Manufactured by the National Mfg. Company, Lincoln, Nebraska.



vious article. An anastigmat lens was used (cost approximately \$25.00).

Four No. 1 photo-flood bulbs, placed close together in the same plane, were used as the light source. A ground-glass plate was used as before to make the light even and diffused. The section of the apparatus con-

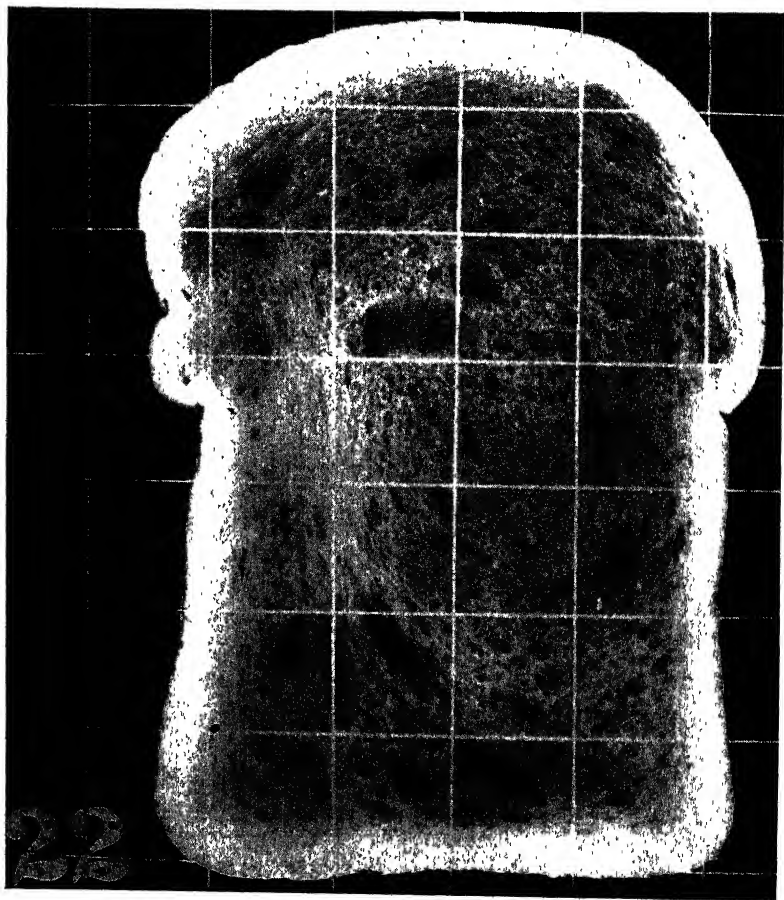


Fig. 1. "Photo-record" of a hand molded loaf of commercial bread, illustrating use of numerals and  $\frac{1}{4}$ -in.-mesh screen

taining the photo-flood bulbs was lined with heavy white paper with a mat surface; a reflector was not used. This light source contributed greatly to the improvement achieved in the pictures of cake. The fogging noticed around the edges of the pound cake shown in Figure 11 of the previous article is mainly due to the relatively long exposure

necessary for the yellow light passing through the yellow cake to affect the photographic paper. By using the photo-flood bulbs this exposure can be shortened so that "fogging" is practically eliminated.

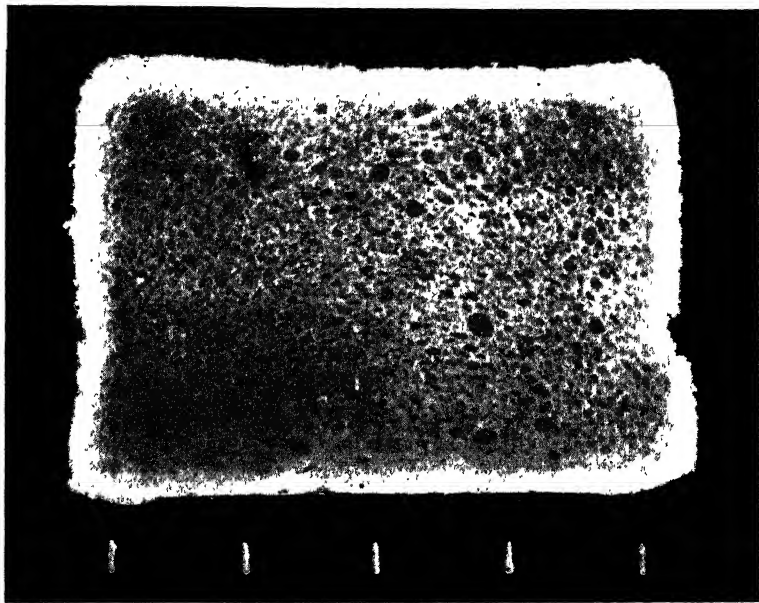


Fig. 2. "Photo-record" of angel food type cake. Thickness of slice  $\frac{1}{16}$  in. Markings at bottom represent  $\frac{1}{16}$  in.

### Photographic Paper

The paper used was Eastman kodabrom, F-No. 2, smooth, glossy, single weight (corresponding papers can be obtained in Agfa and Defender makes). This paper was found to work very well for cakes, including yellow ones, and equally well for bread. Whether this paper is superior to the more "contrasty" papers used for making the pictures in the previous article is perhaps an individual problem. The less contrasty papers yield softer tones and perhaps are more suitable for commercial work. For experimental work, where one wants the defects to be exaggerated, the author favors the contrast papers.

### Numbering the "Photo-Records"

The "photo-records" can be numbered simply by placing the proper numeral on the glass tray which holds the slice of bread or cake. The numerals can be cut out of any non-transparent material. The numbers

shown in Figures 1, 3, and 5 are stamped from ordinary galvanized iron,  $\frac{1}{2}$  inch high, and are obtainable from a hardware store.

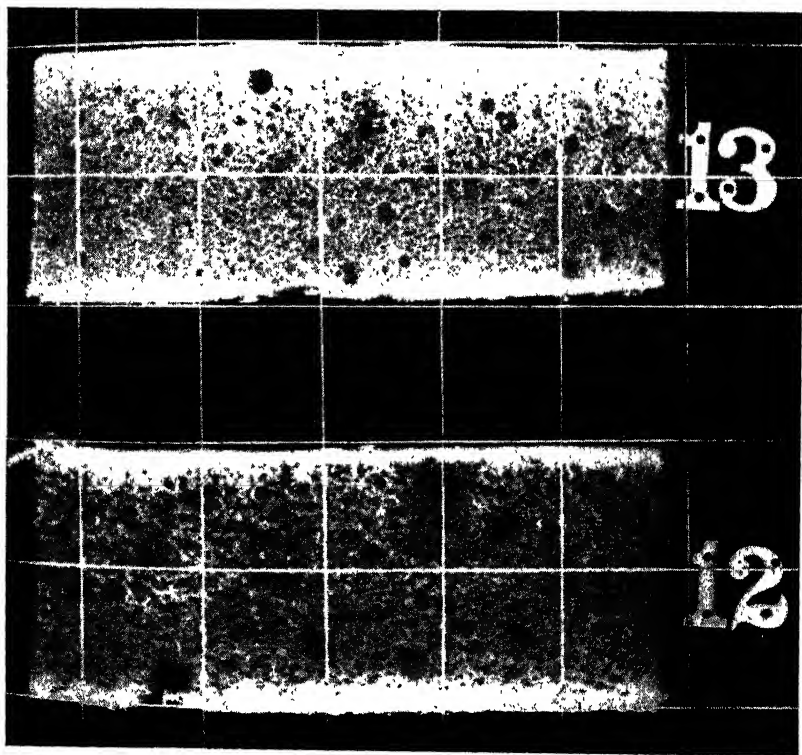


Fig. 3. "Photo-records" of two types of ordinary yellow layer cake. Thickness of slice  $\frac{1}{16}$  in.

### Mesh Screen

A wire-mesh screen placed over the article being photographed is frequently used so that the degree of enlarging or reducing can be determined. Such a screen can be used for "photo-records" also; however, it must be constructed of very fine wire or thread. The one used for the illustrations in this article (Figures 1, 3, and 5) was simply constructed by screening strong thread, No. 25, on a wooden frame by means of tacks.<sup>3</sup> The openings are  $\frac{3}{4}$ -inch square. The screen is placed over the slice of bread or cake before photographing. Figure 1 shows this in use with bread.

<sup>3</sup> It has been suggested that finer thread or wire would improve the pictures by producing a network that would not be so prominent.

### Cake

With cake, "photo-records" are limited to use with light (white and yellow) cakes. Attempts to apply the method to the darker cakes have not been successful. In order to obtain good pictures it is necessary to have the slice of cake thin. For the more porous cakes, such as angel food, a thickness of  $\frac{5}{16}$  inch is about maximum (Fig. 2); for the ordi-

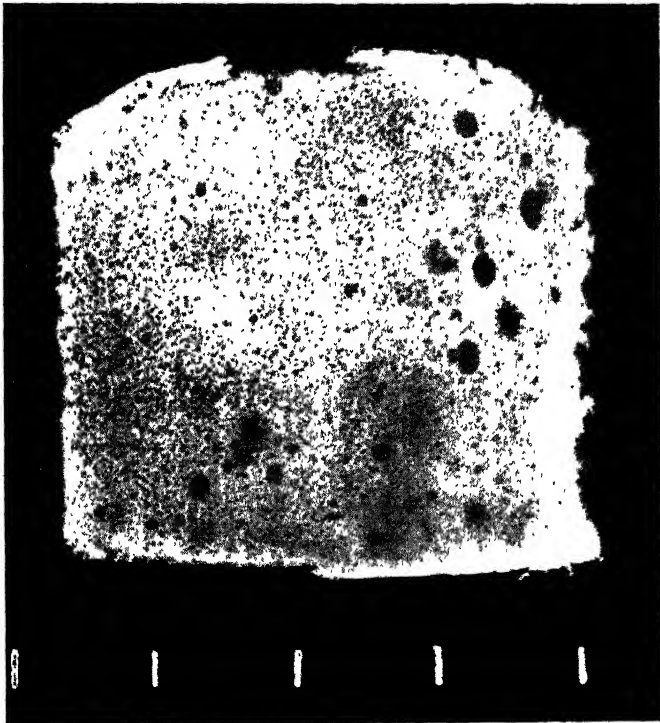


Fig. 4. "Photo-record" of deep yellow pound cake. Thickness of slice  $\frac{1}{8}$  in. Markings at bottom represent  $\frac{1}{4}$  in.

nary yellow layer,  $\frac{3}{16}$  inch is about maximum thickness (Fig. 3); for compact, deep-yellow pound cake,  $\frac{1}{8}$  inch is optimum thickness; however,  $\frac{3}{16}$  inch will serve fairly well (Fig. 4 and 5); and for white high-ratio layer,  $\frac{1}{4}$  inch is about maximum thickness (Fig. 6).

These data on thickness will serve as a guide for most cakes. The limit of thinness to which a cake can be sliced is controlled by its crumbliness; nevertheless, it has been possible to slice almost every cake, of the many tried, thin enough to obtain a good picture. A slicer of the

type used for slicing cold meats was used for slicing the cake; however, a mitre box serves fairly well.

No attempt was made to adjust the thickness of the slices of different cakes so that a uniform time of exposure could be used. Instead, the

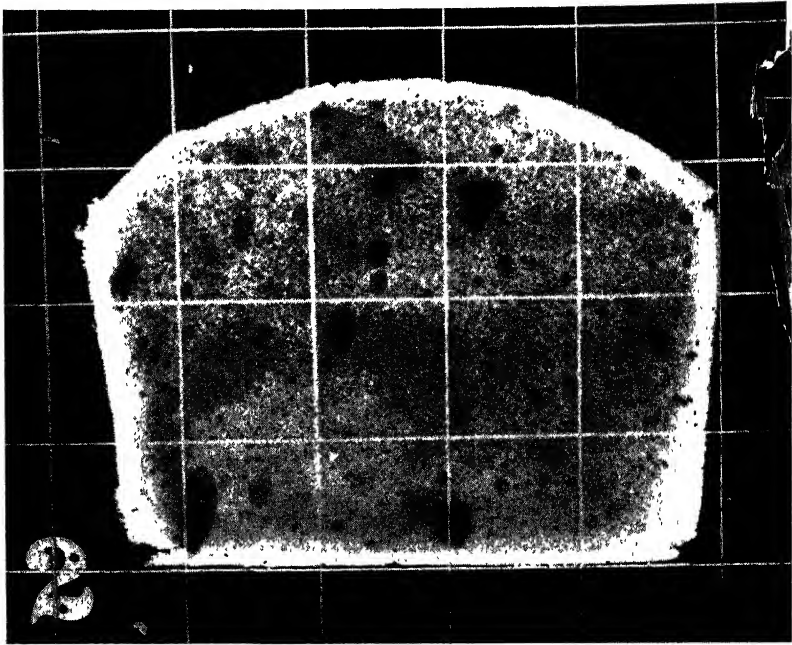


Fig. 5. "Photo-record" of light yellow pound cake. Thickness of slice  $\frac{3}{16}$  in.

time of exposure was adjusted for each different slice. After some experience this can be determined fairly well by holding the slice up to a constant light source. A photoelectric exposure meter serves very well for this purpose when placed over the slice of cake. It also can be used for bread.<sup>4</sup>

A review of the previous article makes the examples of cake shown here self-explanatory.

<sup>4</sup> In the case of white bread, a variation of from  $\frac{3}{16}$  inch to  $\frac{1}{8}$  inch in the slice thickness made only a small difference in the time of exposure.

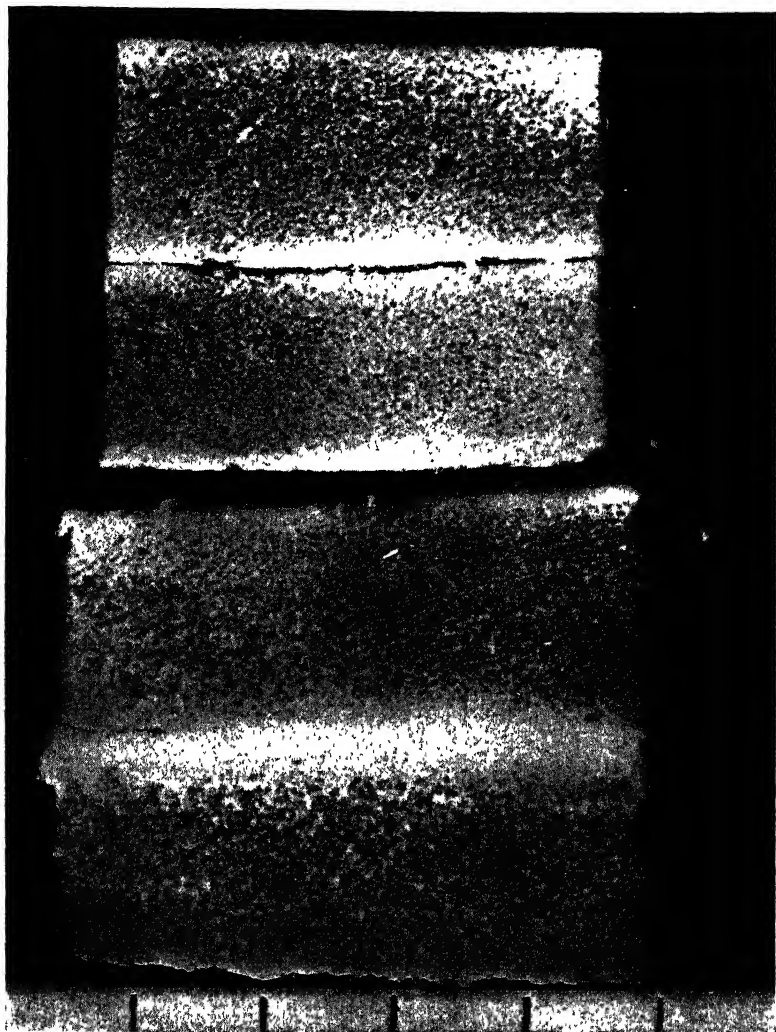


Fig. 6. "Photo-record" of white high-ratio cake. Thickness of slice  $\frac{3}{4}$  in. The light streak in center of the bottom picture is due to dark filling. Markings at bottom represent  $\frac{3}{4}$  in.

#### Acknowledgment

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# MODIFICATION OF THE "SWELLING POWER" TEST FOR THE STALING OF BREAD

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Methods of measuring the rate of staling of bread in terms of definite figures are desirable and necessary. Without them it would be impossible to determine with exactness whether special additions to the dough, special dough treatments, etc., help the keeping properties of the resulting bread. The methods already developed are outlined below. A method having ease of operation, reproducibility, and rapidity of obtaining results is to be herewith recommended.

Lehmann (1894) observed that the crumb of bread, on staling, lost some of its power to swell when immersed in water; that is, he found that fresh bread crumb will swell more than stale bread crumb. Katz (1928) states that Balland was the first to show that the swelling power of bread decreases during staling and that this change can be traced to the starch. Katz (1915, 1917, 1928), on the basis of observations of Balland, Lindet (1902), and Lehmann, has used the swelling-power test for the measurement of the staleness of bread, and has recommended it highly.

According to Katz this method of measuring staleness is more satisfactory for general purposes than the method based on measuring the quantity of soluble amylose (1917, 1928), or on measuring the compressibility (termed hardness by Katz) of the crumb (Katz, 1917, 1928; Platt, 1930; and Bailey, 1930). Measurement of the quantity of soluble amylose is time-consuming, and carbohydrates other than those derived from wheat starch interfere to a small extent. Katz's comment on the above methods is as follows: ". . . the method based on determination of the volume of the decantate [swelling power] is the method best adapted to insure safely reproducible figures. All other methods, even if they seem more rational, involve the objection that they easily develop irregularities in the findings."

Katz has worked with the compressibility method quite extensively and Platt (1930) remarks that "Katz discontinued work by this method in favor of the swelling method, which he recommends in preference. Why this is so, is not clear." Platt points out five advantages of the compressibility method; however, the writers agree with Katz that the main disadvantage is the difficulty of obtaining reproducible results.<sup>1</sup> This is due to the fact that any variation in the

<sup>1</sup> Unpublished observations.

cubical size of the piece of crumb, drying of the outside surface of the crumb, dense spots or dense streaks, holes, and temperature cause variations in the results. This means that several loaves must be tested from each batch and the results averaged.

Katz (1934b)<sup>2</sup> has found that bread shows the same changes in x-ray pattern on staling as do starch pastes. The change in the swelling power runs parallel with the changes in x-ray pattern. Thus the swelling-power method, as do the other methods, measures the changes that take place in the starch during staling. It is also pointed out that the x-ray method is more accurate than the swelling-power method as the sediment is often a bit uneven.

Alsberg (1936) reports that Lopin found an improvement in the swelling-power method by using a 25% alcohol solution instead of water. He reports that this causes sedimentation to occur more rapidly and readings are sharper. According to observation by the writers, this procedure only slightly improves the unevenness which often occurs,<sup>3</sup> and judging from the few experiments which were made the variation in the cubic centimeters of sediment between fresh and stale bread is less than when water alone is used.

Katz (1917, 1928) has demonstrated that an excess of water (as is present with the swelling-power method) fixes the degree of staleness of bread; that is, there is no further staling after the bread crumb is in solution. Thus the sediment can be measured on the same sample as many times as is desired. Also, a sample of crumb may be taken when desired, mixed with water in a stoppered flask, and kept until it is convenient to make the determination.

Karacsonyi (1929) has remarked about the method as follows: "Although we get easily measured differences between fresh and stale bread with this method, its chief drawback is that the determination requires a whole day, and, compared with an eventual viscosimetric method, one must concede the preference to the latter."

Karacsonyi prepares his suspension by a method similar to that of Katz; however, instead of letting it stand for 24 hours, he immediately measures its viscosity with an Ostwald viscosity pipette.<sup>4</sup> Accurate control of the temperature is necessary; thus a thermostat must be used. It is stated that the whole determination can be completed in 40 minutes. Only values for fresh bread and bread 48 hours old are given.

After trying this method Katz remarked that at times it is irregular,

<sup>2</sup> J. R. Katz and co-workers, *Z. physik. Chem.*, A, series of papers.

<sup>3</sup> This unevenness is much more evident in bread which has been frozen. Work has been carried on in these laboratories on "frozen bread" and a way of overcoming this unevenness was found in the method described in this paper.

<sup>4</sup> Both this and the Katz swelling-power method are listed in *Cereal Laboratory Methods*, pp. 86-87, published by A. A. C. C. (1935).



and that it is difficult to determine the relation between the viscosity found and the swelling sought.

Stellar and C. H. Bailey (1938) made a rather extensive investigation of factors which affect the staling of bread. They used the compressibility, sediment volume (swelling power), and viscosity methods concurrently in their investigation. They conclude that, "Compressibility and viscosity measurements of staleness were more consistent and uniform than the data obtained by the sedimentation method. . . . The sedimentation test is not sensitive to minor changes in the condition of the bread and is subject to error due to faulty settling of the crumb."

Although the swelling-power method is accepted as one of the best tests for staleness, Katz, Alsberg (1936a), and L. H. Bailey (1930) have found that it does not show that those procedures of manufacture which the baking trade believes prolongs the life of bread, delay the aging of the starch granules of the crumb. Alsberg comments that "It cannot be doubted that some of these practical procedures do have the effect claimed for them. The explanation probably is that the sedimentation test tells the state of the starch correctly but that this is probably not the whole story."

However, until a better method is developed, the swelling-power method will undoubtedly continue to meet with favor.

The swelling-power method, as described by Katz, has been used in this laboratory. Trouble has been encountered in getting the crumb through the fine bolting cloth, especially in that the cloth breaks very easily with continued rubbing and often the sediments are so uneven that accurate readings cannot be made. Variations of as much as 6 cc. have been noted at times between the maximum and minimum of the sediment surface, with an average of about 4 cc. In order to shorten the time necessary for a determination and minimize the above-mentioned disadvantages, the following work was undertaken.

### Experimental

Katz, in a popularly written article (1934c) mentions that a baker can use a metal sieve instead of the bolting cloth. A brass-frame, 200-mesh sieve 5 inches in diameter has been found to work satisfactorily. The sieve has 78.7 openings per centimeter, and the bolting cloth recommended by Katz has 80 openings per centimeter. The sieve has the advantage of fitting snugly on top of a two-liter pyrex beaker, which serves to catch the washings. The use of the sieve in general has been much more satisfactory than the bolting cloth; in addition the sieve is durable and saves considerable time. However,

this procedure did not eliminate the unevenness in the sediment which often developed.

Since the method is based on sedimentation there seemed to be no reason why the centrifuge would not be helpful in correcting this unevenness. Katz (1934d) has mentioned the use of the centrifuge but does not tell how he used it. However, he remarks: "The method . . . did not work as satisfactorily [as the ordinary method] and was much more complicated." Nevertheless, the writers have experimented with it and have found that the centrifuge can be used satisfactorily.

The wheel of the centrifuge had a diameter of  $10\frac{1}{2}$  inches. A few preliminary experiments indicated that a speed of 1400 r.p.m. would be very satisfactory. This speed has been used throughout the work. The effect of temperature was investigated and variations from  $18^{\circ}$  to  $26^{\circ}$  C. were without effect on the results. Two standard tubes were tried:

- A A 30-cc. conical sediment tube graduated in 0.1-cc. divisions from 0 to 3 cc., in 0.2-cc. divisions from 3 cc. to 10 cc., and in 0.5-cc. divisions from 10 cc. to 30 cc.
- B A 50-cc. conical sediment tube graduated in 0.5-cc. divisions from 0 cc. to 10 cc. and 1.0-cc. divisions from 10 cc. to 50 cc.

The tubes must be thoroughly clean and dry before filling. They are filled from the suspension in the 250-cc. graduate (used in Katz method) after it has been made up to the mark. The solution in the graduate must be shaken thoroughly just prior to filling the sediment tube.

The effect of time of centrifuging on the volume of sediment was studied first. Some typical results are given in Table I and plotted in Figure 1. The sediment in Tube A (30-cc. tube) is more easily measured because of the finer degree of calibration. The surface of the sediment when the 30-cc. tube was used was even and did not tend to develop irregularities as easily as with the 50-cc. tube. Since the amount of sediment drops to a minimum and ceases to decrease, determinations on the rate of staling should be made at a time when the amount of sediment is not varying. This was not done because in this region the surface of the sediment had a tendency at times to become uneven, especially with the 50-cc. tube. It seemed better to centrifuge for 2 or 4 minutes and control the time of centrifuging as accurately as possible. Since there is a more rapid change between the second and fourth minutes of centrifuging in the amount of sediment with the 50-cc. tube than with the 30-cc. tube, the 30-cc. tube is recommended. Nevertheless, the 50-cc. tube can be used and typical results are given in Table II and plotted in Figure 2.

TABLE I

EFFECT OF TIME CENTRIFUGED ON CUBIC CENTIMETERS OF SEDIMENT AT 4, 24, AND 70 HOURS OUT OF OVEN (WHITE BREAD, MEDIUM FORMULA, WRAPPED IN WAX)

Age of bread	Time in centrifuge	Sediment, tube A		Sediment, tube B	
		Separate determinations	Average	Separate determinations	Average
Hrs.	min.	cc.	cc.	cc.	cc.
4	2	2.70, 2.72	2.71	4.50, 4.30	4.40
4	4	2.60, 2.60	2.60	4.30, 4.20	4.25
4	6	2.52, 2.55	2.54	4.00, 4.15	4.08
4	8	2.50, 2.49	2.50	3.95, 4.10	4.03
4	10	2.50, 2.50	2.50	3.90, 4.10	4.00
4	12	2.50, 2.50	2.50	3.90, 4.10	4.00
24	2	2.30, 2.30	2.30	3.65, 3.80	3.73
24	4	2.20, 2.25	2.23	3.50, 3.65	3.58
24	6	2.15, 2.20	2.18	3.50, 3.60	3.55
24	8	2.15, 2.20	2.18	3.45, 3.55	3.50
24	10	2.12, 2.18	2.15	3.45, 3.55	3.50
24	12	2.10, 2.13	2.12	3.45, 3.55	3.50
70	2	2.10, 2.19	2.15	3.50, 3.35	3.43
70	4	2.05, 2.10	2.08	3.50, 3.30	3.40
70	6	2.00, 2.09	2.05	3.45, 3.25	3.35
70	8	2.00, 2.07	2.04	3.42, 3.24	3.33
70	10	2.00, 2.03	2.02	3.40, 3.22	3.31

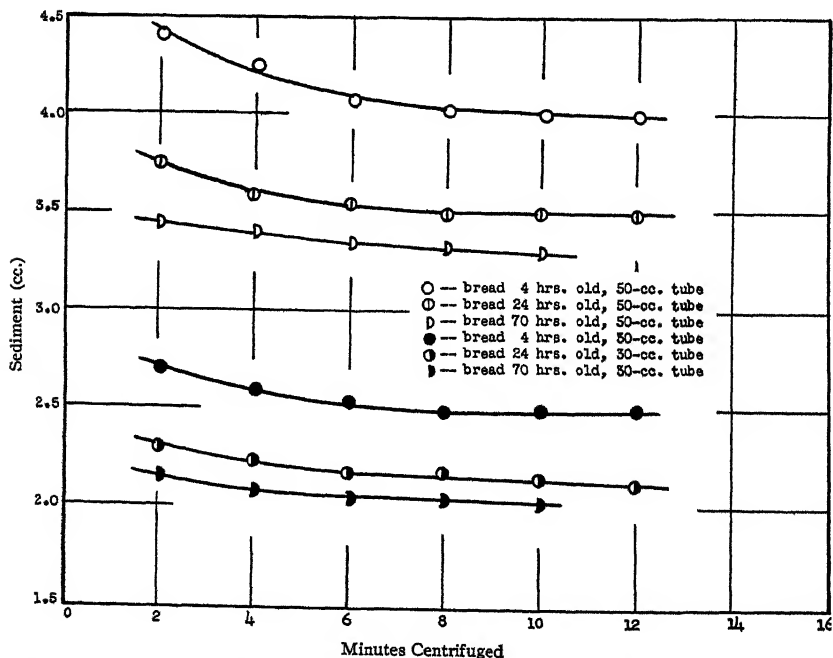


Fig. 1. Effect of time centrifuged on cubic centimeters of sediment at 1400 r.p.m.—bread of various ages, sediment tubes of two sizes.

TABLE II

EFFECT OF AGE OF BREAD NO. 2 (WHITE BREAD, WRAPPED IN WAX, MEDIUM-RICH FORMULA) ON CUBIC CENTIMETERS OF SEDIMENT (50-CC. TUBE USED)

Age of bread	Sediment, 4 min. in centrifuge		Sediment, 8 min. in centrifuge	
	Separate determinations	Average	Separate determinations	Average
Hrs.	cc.	cc.	cc.	cc.
$\frac{1}{8}$	4.95, 4.95, 4.95, 4.95	4.95	4.75, 4.75, 4.75, 4.75	4.75
$2\frac{1}{2}$	4.80, 4.80	4.80	4.55, 4.55	4.55
7	4.50, 4.50	4.50	4.25	4.25
$19\frac{1}{4}$	3.75, 3.75	3.75	3.65, 3.65	3.65
26	3.60, 3.65, 3.55	3.60	3.50, 3.60, 3.50	3.53
46	3.45, 3.50	3.48	3.40, 3.48	3.44
63	3.30, 3.30	3.30	3.25, 3.25	3.25

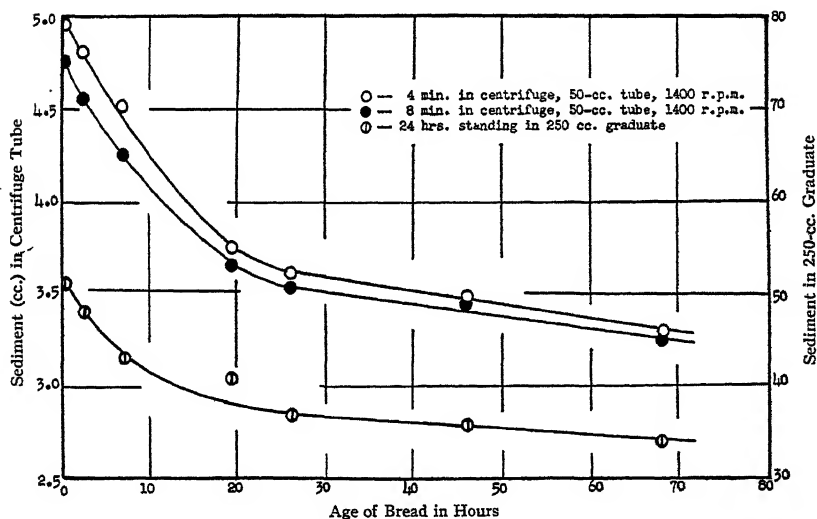


Fig. 2. Effect of age of white bread (medium-rich formula) on sediment.

It will be noted that the results of consecutive determinations agree exceptionally well; however, it must be remembered that this tube cannot be read as accurately as the 30-cc. tube. All the remaining results reported were obtained with the 30-cc. tube. Table III and Figure 3 give typical results obtained for white bread made from a rather rich formula. Determinations also have been made on rye bread. Table IV and Figure 4 are for rye bread (15% dark rye flour). Sediment measurements for the breads reported in Tables II, III, and IV were also made by the Katz method and are reported in Table V. The results are plotted along with the corresponding centrifuge measurements.

TABLE III

EFFECT OF AGE OF BREAD NO. 3 (WHITE BREAD, UNWRAPPED, RICH FORMULA)  
ON CUBIC CENTIMETERS OF SEDIMENT (30-CC. TUBE USED)

Age of bread	Sediment, 2 min. in centrifuge		Sediment, 4 min. in centrifuge	
	Separate determinations	Average	Separate determinations	Average
Hrs.	cc.	cc.	cc.	cc.
$\frac{3}{4}$	3.00, 2.90	2.95	2.80, 2.75	2.78
4	2.70, 2.70	2.70	2.55, 2.55	2.55
7	2.65, 2.65	2.63	2.50, 2.50	2.50
$9\frac{1}{2}$	2.60, 2.59, 2.58, 2.59	2.59	2.45, 2.46, 2.46, 2.44	2.45
12	2.50, 2.50, 2.51, 2.51	2.51	2.35, 2.35, 2.35, 2.37	2.36
26	2.30, 2.30, 2.30, 2.32	2.30	2.20, 2.22, 2.20, 2.15	2.19
47	2.20, 2.10, 2.20, 2.10	2.15	2.09, 2.10, 2.10, 2.18	2.12
70	2.10, 2.19	2.15	2.10, 2.05	2.08

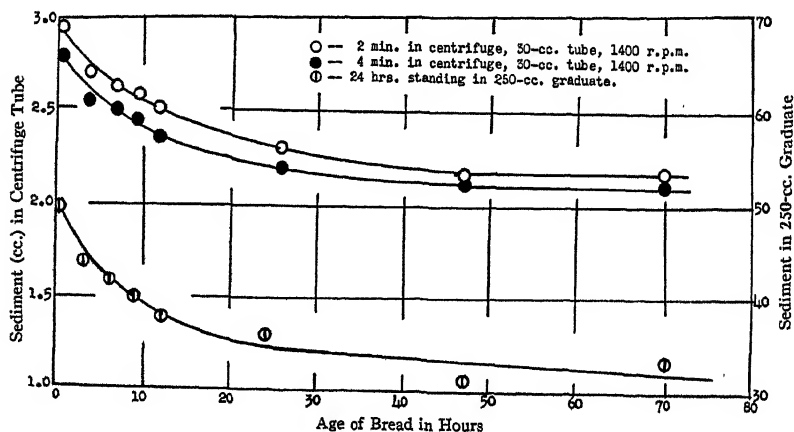


Fig. 3. Effect of age of white bread (rich formula) on sediment.

Throughout the figures it will be noted that the curves determined according to Katz are similar in shape to those determined by the centrifuge method, especially those made with the 30-cc. tube. In general, then, the rate of staling is undoubtedly the same when recorded by either method.

It appears that more points fall off the curve when determined by the Katz method than with the centrifuge method. However, it should be emphasized that all of the results representing one determination were taken from the same loaf of bread. Determinations at different ages of bread were made on separate loaves from the same batch. It was thought that the accuracy might be improved by using sediments from several different loaves (same age, from the same

TABLE IV

EFFECT OF AGE OF BREAD NO. 4 (RYE BREAD, 15% DARK RYE, UNWRAPPED) ON CUBIC CENTIMETERS OF SEDIMENT (30-CC. TUBE USED)

Age of bread	Sediment, 2 min. in centrifuge		Sediment, 4 min. in centrifuge	
	Separate determinations	Average	Separate determinations	Average
Hrs.	cc.	cc.	cc.	cc.
$\frac{3}{4}$	3.05, 3.00	3.03	2.85, 2.80	2.83
4	2.88, 2.89	2.89	2.70, 2.70	2.70
7	2.80, 2.79	2.80	2.67, 2.66	2.67
$9\frac{1}{2}$	2.75, 2.75, 2.75, 2.76	2.75	2.62, 2.63, 2.61, 2.63	2.62
12	2.68, 2.69	2.69	2.53, 2.53	2.53
26	2.50, 2.50, 2.50, 2.48	2.50	2.30, 2.35, 2.30, 2.30	2.32
47	2.38, 2.32, 2.30, 2.32	2.33	2.29, 2.29, 2.20, 2.22	2.25
70	2.25, 2.30, 2.29, 2.30	2.29	2.20, 2.15, 2.20, 2.20	2.19

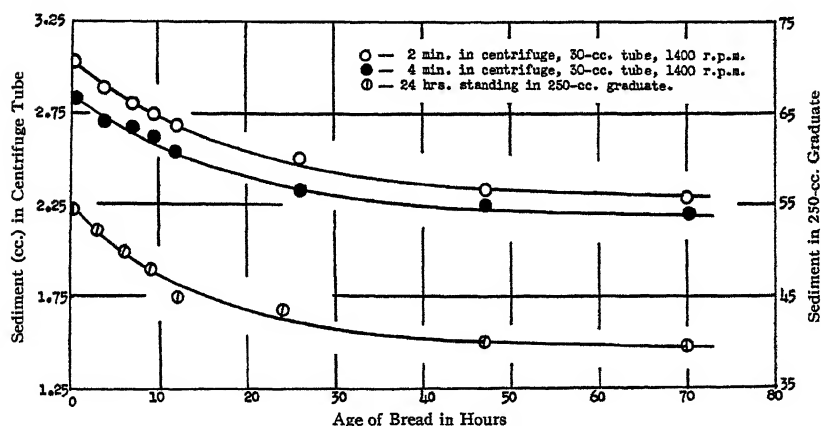


Fig. 4. Effect of age of rye bread (15% dark rye) on sediment.

TABLE V

SEDIMENT (AVERAGE TWO DETERMINATIONS) DETERMINED AS PER KATZ FOR BREADS GIVEN IN TABLES II, III, AND IV

Bread No. 2 (Table II)		Bread No. 3 (Table III)		Bread No. 4 (Table IV)	
Age	Sediment	Age	Sediment	Age	Sediment
Hrs.	cc.	Hrs.	cc.	Hrs.	cc.
$\frac{1}{8}$	51.0	$\frac{1}{4}$	50.0	$\frac{1}{4}$	54.5
$2\frac{1}{2}$	48.0	3	44.0	3	52.5
7	43.0	6	42.0	6	50.0
$19\frac{1}{4}$	41.0	9	40.0	9	48.0
26	37.0	12	38.0	12	45.0
46	36.0	24	36.0	24	43.5
68	34.0	47	31.0	47	40.0
		70	33.0	70	39.5

batch) and the results averaged as was done by Platt (1930) in connection with compressibility measurements. A few experiments indicated that this was true in a few cases, especially when the Katz method was used. However, reliable results can be obtained by making up only one preparation in the 250-cc. graduate and the transferring two aliquots of this into the centrifuge tubes.

Considering the results from the 30-cc. tube or from the Katz method, it is seen that the type of curve depends on the type of bread. The curves for the rye bread start just as high and fall off less gradually than those for white bread, thus substantiating the fact that rye bread stales less rapidly than white. The results herein obtained using the Katz method are very similar to those reported by Katz himself.

It has been mentioned that irregularities in the surface of the sediment with the Katz method (using 250-cc. graduate) have been 4 cc. on the average. Assuming that the correct result can be approximated to within 2 cc. and considering the maximum amount of sediment to be 50 cc., we see that this introduces an error of 4%. Assuming that this is the only reason for variations in separate determinations, the minimum error that would often be present would be 4%.

In the centrifuge modification, there is practically no error due to irregularities in the surface of the sediment, for the surfaces are almost always even after centrifuging for 2 or 4 minutes. However, from the tables it will be seen that the maximum variation in readings with the 30-cc. tube is 0.1 cc. Considering the maximum amount of sediment to be 3.00 cc., the maximum error is 3.3% as compared to a minimum error of 4% for the Katz method.

### The Modified Method in Brief

Ten grams of bread crumb, taken from near the center of the loaf, is weighed to the nearest 0.1 gram and placed on a brass-frame, 200-mesh sieve of 5 inches in diameter; the sieve in turn is placed on a 2-liter pyrex beaker which serves to catch the washings. The bread is moistened with water<sup>5</sup> at  $20 \pm 2^\circ \text{C}$ . The sieve is transferred to a second beaker and the bread rubbed through with the forefinger and the second finger. The washings from the first beaker are used to moisten the crumb as the rubbing is continued. Water is added and rubbing is continued until all of the bread has passed through the sieve. The sieve is then placed back on the first beaker and the entire mass is washed back through the sieve. Care must be taken that the volume is not over 250 cc. The suspension is then transferred to a 250-cc. graduate<sup>6</sup> and the solution made up to 250 cc. The graduate is

<sup>5</sup> Ordinary tap water of about medium hardness has been the most satisfactory in many cases. This is especially true of bread which has been frozen.

<sup>6</sup> Three drops of toluene should be added to prevent fermentation if the sediment suspension is not to be centrifuged immediately.

shaken well and a thoroughly clean centrifuge tube (type A, 30 cc., described above) is filled immediately. The tube is then centrifuged for 2 to 4 minutes <sup>7</sup> (time must be constant) at 1400 r.p.m., when wheel of centrifuge is 10½ inches in diameter. The time and speed can be altered and standardized for wheels of different diameters.

### Advantages and Uses

The above modifications of the "swelling-power" method offer advantages in that the modified method is more convenient, requires less time for a determination (a determination can be made in about 30 minutes as compared to 24 hours for the Katz method), readings can be made more accurately, and the results are very reproducible.

For the benefit of readers other than technicians, the authors wish to point out that one important consideration in any physical or chemical method of measuring staleness is its correlation with actual consumer judgment. Although these methods are of great importance, since they eliminate the human element, a few researches in the past have shown that results obtained by some of these methods do not agree with those of human beings. Reservation is made, pending further research, as to whether the modified method described in this paper gives measurements of staleness which can be correlated with the results of human observers.

It is hoped that this method will enable baking technologists to more easily tell the baker what ingredients, what processes of baking, and what methods of storage will keep his products fresh for the longest periods of time.

### Summary

Various methods for determining the staleness of bread have been enumerated. The swelling-power method has been modified in that a centrifuge is used in determining the amount of sediment and a 200-mesh sieve is used instead of bolting cloth. This makes the method more convenient and it requires less time.

### Acknowledgment

The authors wish to express their sincere appreciation to members of the baking and allied industries for reviewing the manuscript.

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<sup>7</sup> Readings may be taken at both 2 and 4 minutes which when compared with standard curves for 2 and 4 minutes will serve as a check on each other.



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# THE BIOLOGICAL VALUE OF THE PROTEINS OF RICE AND ITS BY-PRODUCTS<sup>1</sup>

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Proteins are not of the same value in nutrition because of the variations in their amino acid content, and the importance of the quality of proteins is universally recognized. A quantitative measure of protein values is obtained by determining the biological value, for which estimation several methods, such as nitrogen balance, growth, or nitrogen storage, are available.

Mitchell and Hamilton (1929) state that values obtained in experiments with pigs and chickens are quite similar to those obtained with rats. The ruminants are excepted, because the microorganisms of the paunch feed on the non-protein nitrogenous material and distinctly modify its biological value.

The purpose of this investigation was to evaluate the proteins of whole brown rice, white polished rice, rice bran, and rice polish for the functions of maintenance and growth.<sup>2</sup> The material was purchased from a local mill and was supposed to be representative of products for human consumption and for feeding pigs and poultry.

A study of the literature revealed that Mitchell (1924), employing the nitrogen-balance method, found an average biological value of 86 for whole rice fed at a 5% protein level, and of 68 for rice bran at a 10% protein level.

Tsan-Wen Li (1930) reported an average value of 79 for a Chinese variety fed at a 10% protein level, and quite recently Basu and Basak (1937) found a value of 80 for polished rice of the Aman variety and also of the Aus variety. Their polishings had an average value of 69 and 68 respectively, at a 5% protein level.

In this paper results obtained with a growth and with a nitrogen-balance method are reported, with the rat as the experimental animal. The compositions of the diets used for these experiments are given in Table I.

## Growth Experiments

A growth comparison of the relative protein values of whole rice and white polished rice at a 5.5% protein level (ration Nos. 1 and 2,

<sup>1</sup> Research paper No. 480, Journal Series, University of Arkansas.

<sup>2</sup> Blue Rose variety obtained from the Cooperative Rice Growers' Assoc., Stuttgart, Ark.

Table I) was made by the modified paired-feeding method of Mitchell, Burroughs, and Beadles (1936). The principle of Osborne and Mendel (1916) that "the only strict basis for comparisons is afforded by experiments in which the animals receive the same amount of food during the same period of time and make the same gains in weight," has been used in his technique. This was accomplished through the use of a nitrogen-free ration (No. 12, Table I), varying amounts of which were fed with the protein ration that was better utilized.

TABLE I  
COMPOSITION OF THE RATIONS

Number and name of ration	N	Salt mixture <sup>1</sup>	Butter-fat	Cellu flour	Dried egg	Starch	Purified sucrose
	%	%	%	%	%	%	%
1. Whole rice (77.0%)	0.92	4.0	8.0	—	—	11.0	—
2. Polished rice (88.0%)	0.92	4.0	8.0	—	—	—	—
3. Rice bran (67.0%)	1.32	4.0	8.0	—	—	21.0	—
4. Rice polishings (65.0%)	1.32	4.0	8.0	—	—	23.0	—
5. Casein (7.0%)	0.93	4.0	10.0	4.0	—	65.0	10.0
6. Rice polishings (37.0%)	0.92	4.0	10.0	—	—	39.0	10.0
7. Rice bran (34.0%)	0.92	4.0	10.0	—	—	42.0	10.0
8. Whole-milk powder (31.2%)	1.31	4.0	10.0	4.0	—	40.8	10.0
9. Skimmilk powder (23.7%)	1.31	4.0	10.0	4.0	—	48.3	10.0
10. Polished-rice protein extract (51.0%)	1.36	4.0	10.0	4.0	—	21.0	10.0
11. Standardizing ration	0.60	4.0	10.0	4.0	4.0	68.0	10.0
12. Nitrogen-free ration	—	4.0	10.0	4.0	—	72.0	10.0

<sup>1</sup> Osborne and Mendel No. 351.

Nine pairs of rats were used in this comparative study. Litter mates of the same weight and sex were employed, the initial weight being about 50 grams. Individual food-consumption records were kept and the animals were weighed twice a week, when adjustments were made for the intake of the nitrogen-containing ration and the ration that was nitrogen free. The nine pairs of rats were killed at the end of 130 days and their body length from mouth to anus was measured. Similar measurements were made at the beginning of the experiments. Differences in increase in body length might be found between the two growth-promoting proteins. The results of this paired-feeding experiment are given in Table II.

In all nine pairs the whole-rice-fed rats utilized their food better than the polished-rice-fed ones, and less nitrogen-containing food was needed by the former for the same gain in weight as was obtained by the latter. A certain intake of nitrogen from the whole-rice ration promoted the

TABLE II  
A COMPARISON OF THE GROWTH-PROMOTING VALUE OF THE PROTEINS OF WHOLE RICE  
AND POLISHED RICE BY A MODIFIED PAIRED-FEEDING METHOD  
Duration 130 days, 5.5% protein intake.

Pair No.	Rat No. and sex	Ration	In-crease in body weight	In-crease in body length	Food intake	Nitro-gen in-take	Ratio	Protein (N X factor) <sup>1</sup> intake	Gain per gram of protein intake
			g.	mm.	g.	g.		g.	g.
1	1 ♀	Whole rice	72	45	866 (50) <sup>2</sup>	7.95	1.00	47.30	1.52
	2 ♀	Polished rice	72	45	916	8.45	1.06	50.27	1.43
2	3 ♀	Whole rice	99	56	965 (80)	8.73	1.00	50.94	1.94
	4 ♀	Polished rice	99	50	1045	9.63	1.10	57.29	1.73
3	5 ♀	Whole rice	87	48	883 (80)	8.16	1.00	48.55	1.79
	6 ♀	Polished rice	87	48	965	8.88	1.09	52.83	1.65
4	7 ♂	Whole rice	82	45	827 (70)	7.64	1.00	45.46	1.80
	8 ♂	Polished rice	82	41	897	8.27	1.08	49.21	1.67
5	9 ♂	Whole rice	101	41	977 (100)	9.02	1.00	53.67	1.88
	10 ♂	Polished rice	101	41	1077	9.93	1.10	59.08	1.71
6	11 ♂	Whole rice	102	40	975	9.01	1.00	53.61	1.91
	12 ♂	Polished rice	102	40	1040	9.59	1.06	57.06	1.79
7	13 ♂	Whole rice	94	52	900 (50)	8.31	1.00	49.44	1.90
	14 ♂	Polished rice	94	46	950	8.76	1.05	52.12	1.80
8	15 ♂	Whole rice	94	42	965 (110)	8.91	1.00	53.01	1.77
	16 ♂	Polished rice	94	41	1075	9.91	1.11	58.96	1.61
9	17 ♂	Whole rice	83	41	925 (50)	8.55	1.00	50.87	1.63
	18 ♂	Polished rice	83	41	975	8.99	1.05	53.49	1.55
		Average, whole rice	—	—	920 (73)	8.47	1.00	—	1.80
		Average, polished rice	—	—	993	9.15	1.08	—	1.66

<sup>1</sup> Factor for rice 5.95.

<sup>2</sup> Amount of nitrogen-free ration consumed.

Mean difference (M), 0.140; standard deviation of differences (S), 0.042; ratio M/S, 3.3; odds, 9,999 : 1; probability, 0.00001.

same gain in weight as a larger intake of polished-rice nitrogen. The ratios between the nitrogen intake of pair mates (Table II, eighth column) indicate that one gram of nitrogen from the whole rice ration was as effective in promoting the functions of maintenance and growth as an average of 1.08 g. of nitrogen from the polished-rice ration. The average gain in weight per gram of protein intake was 1.80 for the animals fed the whole-rice ration and 1.66 grams for those fed the polished-rice ration, with a statistically significant difference of 0.14 gram. Analyzed by Student's method (1908), the probability that chance alone determined the outcome is 0.00001 ( $M = 0.14$ ,  $S = 0.042$ ) with reference to the difference between the average gains obtained. A

TABLE III

A COMPARISON OF THE GROWTH-PROMOTING VALUE OF THE PROTEINS OF RICE BRAN AND RICE POLISH BY A MODIFIED PAIRED-FEEDING METHOD

Duration 112 days, 8% protein intake.

Pair No.	Rat No. and sex	Ration	Increase in body weight	Increase in body length	Food intake	Nitrogen intake	Ratio	Protein (N X factor) <sup>1</sup> intake	Gain in weight per gram of protein intake
			g.	mm.	g.	g.		g.	g.
10	19 ♂	Rice bran	129	45	991	13.03	1.14	78.52	1.64
	20 ♂	Rice polishings	129	51	871 (120) <sup>2</sup>	11.45	1.00	68.12	1.89
11	21 ♂	Rice bran	125	51	1120	14.72	1.25	87.58	1.43
	22 ♂	Rice polishings	125	51	895 (225)	11.77	1.00	70.03	1.78
12	23 ♂	Rice bran	140	47	1018	13.38	1.12	79.61	1.76
	24 ♂	Rice polishings	140	51	908 (110)	11.94	1.00	71.04	1.97
13	25 ♀	Rice bran	103	49	900	12.23	1.30	72.76	1.42
	26 ♀	Rice polishings	103	49	765 (195)	9.40	1.00	55.93	1.84
14	27 ♀	Rice bran	124	53	1084	14.25	1.13	84.78	1.46
	28 ♀	Rice polishings	124	53	954 (130)	12.54	1.00	74.61	1.66
15	29 ♀	Rice bran	111	47	1030	13.54	1.13	80.56	1.38
	30 ♀	Rice polishings	111	49	910 (120)	11.96	1.00	71.16	1.56
16	31 ♂	Rice bran	93	45	900	11.83	1.34	70.38	1.32
	32 ♂	Rice polishings	93	45	670 (230)	8.81	1.00	52.41	1.77
17	33 ♀	Rice bran	122	45	1100	14.46	1.22	86.03	1.42
	34 ♀	Rice polishings	122	46	900 (200)	11.83	1.00	70.38	1.73
18	35 ♂	Rice bran	129	55	1082	14.23	1.21	84.66	1.52
	36 ♂	Rice polishings	129	57	892 (190)	11.73	1.00	69.79	1.85
		Average, rice bran	—	—	1032	13.57	1.20	—	1.48
		Average, rice polishings	—	—	863 (169)	11.35	1.00	—	1.79

<sup>1</sup> Factor for rice 5.95.<sup>2</sup> Amount of nitrogen-free ration consumed.

Mean difference (M), 0.31; standard deviation of differences (S), 0.097; ratio M/S, 3.2; odds, 9,999 : 1; probability, 0.00001.

probability smaller than 0.03 is according to current biometrical practice a criterion of high significance. In four pairs the increase in body length was greater in the whole-rice-fed animals; in the other five pairs the increase was the same for both rats.

A similar comparative study was made of the growth-promoting value of the proteins of rice bran and rice polishings, by exactly the same technique as described above. The protein level was 8% (rations 3 and 4, Table I) and the experiment lasted 112 days. The results of this experiment are given in Table III, from which it can be seen that

in all nine pairs the rice-polish-fed rats needed less nitrogen to promote the same gain in weight than the rice-bran-fed ones. The ratios between the nitrogen intake of pair mates (Table III, eighth column) indicate that one gram of nitrogen from the rice polishings ration was as effective in promoting the functions of maintenance and growth as an average of 1.20 grams of nitrogen from the rice-bran ration. The average gain in weight per gram of protein consumption was 1.79 g. for the animals fed the rice-polish ration and 1.48 g. for those fed the rice-bran ration. The difference of 0.302 g. between these gains was found to be statistically significant when Student's method for the statistical analysis of paired experimental observations was applied ( $M=0.31$ ;  $S=0.097$ ;  $P=0.00001$ ). In five pairs the increase in body length was greater in the rice-polish-fed rats; the increase was the same in the remaining four pairs.

### Metabolism Experiments

Differences in the growth-promoting values of the rice proteins as revealed by the modified paired-feeding method can be explained with the aid of metabolism experiments in which digestibility coefficients and biological values are obtained. The metabolism method of Mitchell (1923) was used in these nitrogen balance studies. Young rats of an initial weight of 60 to 70 g. were employed for the determination of the biological value of rice and its by-products at 5% and 8% protein levels. The experiment consisted of three periods and the test animals were divided into two groups with 4 or 5 animals in a group.

In the first period the test rations were fed to two different groups and in the third period the test rations were reversed for these two groups, while both groups received the standardizing ration (No. 11, Table I) in the second period.

The results of these nitrogen balance studies are summarized in Table IV. Biological values obtained for whole-milk powder and skim-milk powder, casein, and lactalbumin are included for comparison. It can be seen that although whole rice had a somewhat lower digestibility than polished rice (96.5 against 98.0), the biological value of the latter is lower than that of the former, resulting in a better utilization of the whole-rice protein at a 5% level of protein intake and a better rate of growth.

Both digestibility and biological value are lower for the rice bran, compared with those for rice polish at an 8% level of protein intake. Rice polish is better utilized and promotes better growth than rice bran as a result of the greater losses of the nitrogen of rice bran during digestion and metabolism.

TABLE IV  
DIGESTIBILITIES AND BIOLOGICAL VALUES OF THE PROTEINS OF RICE AND ITS BY-PRODUCTS AT TWO PROTEIN LEVELS

Protein	Number of determinations	Average true digestibility	Average biological value
5-6% whole rice	48	96.5±.29	72.7±.34
5-6% polished rice	30	98.0±.18	66.6±.18
5% rice bran	8	77.6±.66	84.9±.63
5% rice polishings	18	91.3±.40	82.9±.96
5% casein	8	98.2±.49	81.5±1.00
8% rice polishings	10	88.7±.60	78.9±.97
8% rice bran	10	83.0±.64	71.9±.78
8% whole milk powder	10	93.5±.41	85.6±1.05
8% skim milk powder	10	93.9±.57	87.8±.71
8% casein <sup>1</sup>	12	99.0±.22	69.5±1.00
8% lactalbumin <sup>1</sup>	15	98.0±.40	84.0±.60
9% polished rice protein extract	8	78.3±.48	66.2±.51

<sup>1</sup> Data from: Digestibility, Metabolism, and Nutritive Value of Lactalbumin, by M. C. Kik, University of Arkansas Bulletin No. 352.

The biological values of rice bran and rice polish at a 5% protein level are higher than those of the whole kernel and of the rice flour and compare very favorably with that obtained for casein at that protein level.

At an 8% level of protein intake, the values are somewhat lower for the proteins of rice bran and rice polish than those obtained for whole-milk powder, skimmilk powder, and lactalbumin, all of which are known to possess a high nutritive value.

### Summary

A growth comparison of the relative protein values of whole rice and white polished rice fed rats at a 5.5% protein level and of rice bran and rice polishings at an 8% protein level was made using a modified paired feeding method. It was found that 1 g. of nitrogen from the whole-rice ration was as effective in promoting the functions of maintenance and growth as an average of 1.08 g. of nitrogen from the polished-rice ration. One gram of nitrogen from the rice-polishings ration was as effective as 1.20 g. of nitrogen from the rice-bran ration. The average gain in weight per gram of protein intake was 1.80 g. for the rats fed whole rice and 1.66 g. for those fed the white polished rice, at a 5.5% protein level. These figures were 1.79 g. for rats fed rice polishings and 1.48 g. for rats fed rice bran at an 8% protein level.

The biological values (Mitchell's method) for whole rice, white polished rice, rice bran, rice polishings, and casein at a 5% protein level were 72.7, 66.6, 84.9, 82.9, and 81.5 respectively. These values were 85.6, 87.8, 69.5, 84.0, 78.9, and 71.9 for whole-milk powder, skimmilk powder, casein, lactalbumin, rice polishings, and rice bran respectively, at an 8% protein level.

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## BOOK REVIEW

**Untersuchungsmethoden für Brotgetreide, Mehl und Brot.** By Paul Pelshenke. Published by Moritz Schafer, Leipzig, Germany. 288 pages. Price R.M. 11.5.

This manual includes a vast amount of material in relatively small space. The analytical and other methods are classified under 163 sections or divisions and many of these are further subdivided to include several methods designed for the same general purpose.

Sampling methods are presented in the first section, followed by a series of physical tests of cereals. Purity, germinating power, and various forms of damage are then covered. Methods for the determination of ash, protein (including the individual proteins of wheat), gluten quantity and quality, fermentation and enzymes, physical properties of dough, carotin and color, acidity and pH, carbohydrates, fats, fiber and pentosans are presented in the order named. This is followed by the description of many special methods adapted to cereals (bread and other cereal products, as well as baking powder), identification of impurities and mixtures, presence of special ingredients, and conformity of products to standards of quality. Evaluation of bread quality is discussed, together with score cards for various bread types. A series of 39 tables is provided which include equivalents of hectoliter weight in pounds per bushel, sugar conversion tables, buffer solutions, sieve dimensions, relative humidity, and other pertinent data.

The book is least complete in the departments devoted to physical and physico-chemical methods. Thus the electrical methods for the estimation of moisture content are dismissed with about eight lines of text. Mechanical dough testing is discussed at some length (about a dozen pages) but no diagrams of equipment or specimen curves are included. In fact the book contains no illustrations whatever, and one regrets the lack of these in certain sections.

In addition to a detailed table of contents facing page 1, the book is provided with a good subject and author index.

The reviewer feels that Dr. Pelshenke has contributed a useful manual, replete with carefully selected and tested methods. There has been a singular lack of such books of methods in the literature of recent years and this manual should be of large service in the laboratories engaged in cereal testing and research.

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## A REVIEW OF THE 1938 LITERATURE PERTAINING TO THE FIELD OF CEREAL CHEMISTRY<sup>1</sup>

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(Read at the Annual Meeting, May 1939)

This is a continuation of the series of similar papers presented at previous meetings by Clinton L. Brooke (1936). Because of the rather short time available for the preparation of this article, it was thought advisable not to select a large number of references, but to discuss the data and conclusions in somewhat greater detail. Let us divide the subject, considering first whole grain, then flour, and finally bread.

During the past year the fumigation of grain has attracted attention. Although carbon disulphide has been in use for this purpose since 1857, its inflammability and odor make it highly objectionable. Furthermore, Cayzer (1938) has shown that carbon disulphide has a decided weakening effect on the gluten, although after storage for two months the flour improved as did the control through natural aging. The carbon-disulphide-treated flours when baked soon after milling gave small "green" loaves by both the malt-phosphate and the malt-phosphate-bromate procedures. The doughs made from the CS<sub>2</sub>-treated flours were of poor handling quality and the texture of the bread was coarse and heavy. The possibility that this is another case of SH oxidation is mentioned.

Cayzer (1938) also tested a mixture of 75% ethylene dichloride and 25% carbon tetrachloride as a fumigant. This mixture was found quite effective in the control of insects and had no weakening effect on gluten quality as shown by the farinograph. Loaf-volume measurements indicated further that there is close correlation between farinograph and baking results.

The conditioning of grain prior to milling is probably the most important technical question now confronting the milling industry. Much of the practical experience of the past and many of the isolated opinions of specialists can now be united to form the beginning of a

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theory of this process. Besides the well-known factors of temperature, moisture, and time, there are unexpected relationships involving the chemical and perhaps the enzymic composition of the grain. Which of these are the most important and which can be neglected in practice? How far do laboratory results help to decide this question?

In any case it seems evident that the utilization of each new crop requires that attention be given to the conditioning of the grain. Ritter (1938) makes the point that the effect of relatively high conditioning temperatures is a neglected but important factor in the milling of grain. Mueller (1938) is of the opinion that no one procedure gives good results with all kinds of wheat.

Issue No. 22, Vol. 75, of *Die Mühle* is a special edition on wheat conditioning. Boulanger (1938) discusses whether the baking and milling qualities are really affected by conditioning, and concludes that they are very much so. Argus (1938) points to the incompleteness of definite knowledge and the present opportunities for misunderstanding between mill and laboratory. Berliner (1938) considers changes in the interior of the grain of the highest importance, particularly partial denaturation of the proteins and modifications of enzyme activity as produced by heat and the chemical effect of the added water. Hübsch (1938) on the other hand is inclined to minimize the importance of enzyme changes, and regards alterations in the viscosity of starch and proteins as of chief interest. This author differs from most current writers in being far from convinced that conditioning grain improves the baking qualities of flour.

Ritter (1938) considers that the enzymes of most probable importance in conditioning are first the starch-splitting ferments  $\alpha$ - and  $\beta$ -amylase and the newly discovered amylophosphatase. Proteinases, peptidases, phosphatases, the enzymes of oxidation and reduction, and in fact most of the other known ferments come in for attention, however. These enzymes are thought to play a double role in the wheat; during ripening they synthesize and during conditioning and baking they break down starch, protein, and other constituents.

It is only a step from the conditioning of wheat to studies on changes during germination. Thus Pulkki and Puutula (1938) found that during the germination of a Manitoba wheat ascorbic acid occurred in the sprouts to an extent far outweighing that found in the rest of the grain. The maximum amount of ascorbic acid found was 0.022% on dry weight. The sprout also contains a substance having considerable proteolytic activity, in fact so much that the proteolytic activity far overcomes the effect of the ascorbic acid on the baking strength. The grain with sprout removed contained most of the amylase.

Kosmina and Romanova (1938) found that up to the fourth or fifth

day of sprouting the gluten content of wheat grain undergoes only a small decrease. In the course of the sprouting the ordinarily smooth and elastic gluten becomes crumbly and is easily torn; accordingly the dough shows poor elasticity and the volume of the bread is reduced.

The cause of these alterations in the gluten quality is said to be the hydrolysis of fat in the sprouting grain, leading to an accumulation of unsaturated fatty acids which exert an influence on the colloidal properties of the gluten. The sprouting process also influences the activation of proteolytic enzymes capable of digesting gluten. The authors, however, regard the effects of fatty acids and proteinase activity as opposing each other, for no liquefaction of gluten was observed until removal of the fatty acids from the flour.

Mounfield (1938) presents the third paper in a series on the proteolytic enzymes of sprouted wheat. He has previously reported the properties of the proteinase and dipeptidase in water extracts of sprouted wheat. The present paper deals primarily with the action of the proteinase on gelatin, ovalbumin, gliadin, glutenin, and gluten as shown by changes in formal titrations. Wheat proteinase was found to hydrolyze gelatin at pH 5.1 and wheat gluten at pH 6, but did not attack ovalbumin and affected isolated glutenin and gliadin only slightly. Activation of wheat proteinase by cyanide was observed when edestin was the substrate but not when gluten was employed.

Of the many interesting studies on flour and its constituents, only a few can be reported here.

Practical importance attaches to a photoelectric colorimeter described by Palmer (1939), which measures color in terms of the percentage of light absorbed in different parts of the spectrum. The surface of the flour is illuminated by light of controlled intensity falling on it at an angle of 45 degrees. The reflected light is filtered through a cell containing copper sulphate solution and is then passed through color filters that are so mounted as to be changed readily. After passing through the filter the colored light falls on the photoelectric cell, which is connected with a reflecting galvanometer. Nine color filters are recommended. The color of the flour can be described in terms of brightness, grayness, and yellowness. The recording of the color values of a flour occupies about five minutes. The instrument can also be used for measuring color in bread. A definite improvement of color was noted in flour after aging for 21 days.

Jørgensen (1938) has continued his work on the effect of bromate in the dough, and has secured additional evidence for the view that the change in gluten is due to inhibiting the action of a papain-like proteinase in the flour. It was found that very low concentrations (0.002 molar) of bromate and iodate strongly inhibited the digestion

of casein by papain, whereas chlorate did not inhibit, even in large amounts. Furthermore, and in contradiction to earlier observations, these inhibitions were not dependent on the type of buffer used. Iodate and bromate, but not chlorate, also inhibit bromelin, and since the presence of these inhibitors also reduces the amount of water-soluble nitrogen that may be extracted from a flour, it follows that they inhibit the flour proteinase as well. The article by Jørgensen, cited here, is one of several papers of great interest to cereal chemists, which appear in the volume of *Carlsberg's Comptes Rendues* commemorating the 70th birthday of S. P. L. Sørensen.

The discovery by Jørgensen in 1935 that ascorbic acid was a good bread improver has increased the interest of cereal chemists in this substance. Ascorbic acid was already known to inhibit the activity of papain, but this cannot be due to oxidation, for ascorbic acid is a strong reducing agent. Jørgensen, in order to explain the effect in dough, advanced the view that it might act as an oxygen carrier, absorbing oxygen from the atmosphere and then in turn oxidizing the flour proteinase. This is in agreement with the remarkable observation of Hopkins and Morgan (1936) that when reduced glutathione and ascorbic acid are mixed in the presence of ascorbic acid oxidase and air, the glutathione is oxidized first, and not until all the glutathione is gone does the ascorbic acid undergo oxidation.

More recently Melville and Shattock (1938) have shown that flour contains ascorbic acid oxidase, and that dehydroascorbic acid is usually a better bread improver than the reduced form. So the connections among ascorbic acid, glutathione, certain oxidative enzyme systems, and the quality of gluten seem well established.

The behavior of flour is of course not the only factor in the quality of the dough. Fermentation is an important part of the baking process, and Hofman and Hanke (1938) have made a recent study on the stimulation of fermentation caused by the addition of organic acids. Farinograph measurements, coupled with observations on the gas generated, have shown that fermentation was stimulated by the addition of about 0.25% lactic acid and 0.13% acetic acid, while addition of butyric acid was without result. With these organic acids it is necessary to use a larger dose of yeast, for the increased acidity may favor the development of wild organisms.

While the enzyme systems of flour and particularly their capacity for modifying the gluten have thus received much attention, the structure and physical properties of both flour protein and starch have been discussed in numerous papers.

Ritter (1939) notes that the intake of water by gluten which has been stretched and then allowed to dry causes a swelling that increases

the thickness of the gluten strands far more than it increases their length. Such observations emphasize the fiber-like structure of flour protein. However, certain soluble proteins of small molecular weight—myosin, for example—can be transformed into fibers, and this seems to show a close connection between proteins of both kinds. It is only necessary to dissolve myosin and reprecipitate it while the particles have a linear motion in order to obtain a fibrous form.

Schmorl (1938) has summarized the present-day knowledge of starch in a chemical formula that seeks to explain the physical property of gel-formation and such chemical facts as the phosphorus content, the capacity for further phosphorylation by a phosphorylizing enzyme, and the difference in hydrolysis produced by the two types of amylase. The modern tendency seems to be to regard the colloidal properties of starch as being due to peculiarities in the chemical build-up rather than to "outside" factors.

Passing at last from flour and dough to bread itself, mention should be made of two of the most important problems in the field, the staling of bread and the flavor of bread.

A paper by Fuller (1938) presents an interesting report on the problem. The author states that bread goes stale in spite of many patent remedies. The work and theories of Platt, Alsberg, Katz, and Ostwald are discussed fully.

A study of the structure and properties of starch appears relevant to the problem. That starch is the fraction of the bread most directly concerned in staling appears to have been definitely established; furthermore, staling is apparently due to a reduction in the hydration capacity of the gelatinized starch which causes a change in the structure of the gel.

Partial disaggregation of the starch molecules occurs on heating, thus altering the proportion of  $\alpha$  and  $\beta$  amylose, and there would appear to be a definite equilibrium between the two forms at any one temperature. It is suggested that to the comparatively slow attainment of equilibrium between the two forms may be attributed the phenomenon of staling. If this is so, then staling can only be affected by a major alteration in the equilibrium proportion of  $\alpha$  and  $\beta$  amylose, such as would be obtained by keeping the bread at an elevated temperature or by altering the state of hydration of the starch present.

One method of altering the state of hydration of the starch is by freezing, another method is by adding soluble solids such as sugar. A third possibility is the discovery of some edible substance that, when added to the starch gel, will prevent molecular reaggregation.

In reference to the flavor question, attention is called to a paper by Maybee (1939). This paper discusses theories of the flavor, odor,

and taste of foods in general. The following subjects are also treated: relation of flavor to constitution; classification of flavor sensations, synthetic flavors, subsidiary flavors developed during storage; the threshold value, sweet, bitter, sour and salty taste, classifications of odor sensations, food-tasting panels; and methods of measurement that can be standardized in the control laboratory.

A paper by Pelshenke (1938) discusses reasons for the decline in bread consumption. The flavor of bread, it is stated, is governed above all by the type. Some countries have bread of a decided yeasty or sour taste, others with a neutral taste. The influence of raw materials and improvers plays an important role in taste and flavor. Flour is almost tasteless, and if the bread is to be pleasing this must come about through other ingredients. In particular, two groups of substances govern the flavor of bread, namely the soluble disintegration products of starch that are formed during fermentation and the products in the crust formed during baking. The main problem is to treat the bread so that these bodies will be produced. Beyond a doubt the speed and the acceleration of the fermentation process can be accomplished only at the expense of the development of flavor. The addition of pleasantly aromatic organic compounds to the dough is impractical as they are volatile and so are lost during baking. Influencing factors such as improvers, ovens, and dough conditions are also discussed.

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## NOMOGRAMS FOR CALCULATING ABSORPTION OF FLOUR AND SEMOLINA <sup>1</sup>

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Where a large number of tests is involved, the arithmetical calculation of flour and semolina absorption from baking and macaroni test data is tedious and time-consuming. These computations have recently been replaced in this laboratory by the use of nomograms. These have proved so convenient that it has been thought worth while to render them generally available and to describe the procedure involved in their construction.

<sup>1</sup> Contribution from the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba. Published as paper No. 158 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.

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### Construction of a Nomogram for Absorption Calculations on Flour Doughs

In the official A. A. C. C. basic baking test (Geddes, 1934), the percentage absorption is computed from the equation:

$$\% \text{ absorption (15\% moisture basis)} = 45.6 + W - (100 - F),$$

where 45.6 represents the cc. of water added per loaf in the form of stock solutions;  $W$  is the cc. of additional distilled water added to produce a dough of the desired consistency; and  $F$  is the weight of flour required to give 100 g. on a 15% moisture basis (85 g. dry matter).

As the figure 45.6 is a constant, the variables in the equation are percentage absorption, volume of added water (cc.), and the difference  $(100 - F)$ . Since  $(100 - F)$  is related to the flour moisture content, it is more convenient to employ the moisture values directly.

In the construction of the graph, the first step is to draw two parallel lines of such length that the desired scale range can be conveniently covered without making the finest subdivisions unduly small; the distance between these parallel lines should be approximately two-thirds of their length. On one is constructed a scale for moisture, and on the other a scale for water added using accurately measured increments of lengths for each 1% flour moisture and each cubic centimeter of added water. Let these respective lengths be designated as  $K_1$  and  $K_2$ . A third axis is then drawn parallel to and between the axes for flour moisture and added water so located that the distance between it and the flour-moisture and added-water scales respectively is in the same proportion as  $K_1$  to  $K_2$ . On this central axis the absorption scale is constructed. As a starting point, a fixed point on the scale is located by joining any two values on the flour-moisture and added-water scales; the correct absorption for this point is calculated by the equation previously given and designated on the scale. From this reference point the absorption scale is laid out, using unit divisions of the length  $\frac{K_1 K_2}{K_1 + K_2}$ .

In this laboratory the flour is weighed out on a 13.5% moisture basis, and a nomogram for use in computing flour absorptions to this basis is reproduced in Figure 1. The reading of the nomogram is simply accomplished by joining with a straight edge the value for flour moisture with that for the cubic centimeters of water added and reading the corrected absorption from the center scale. A celluloid cursor is very satisfactory for aligning the points. The moisture scale ranges from 10% to 15% in increments of 0.1%, and the added-water scale from 3 to 23 cc. in increments of 0.2 cc. The percentage of

absorption therefore varies from 44 to 70 and the graduations permit of accurate interpolation to 0.1%.

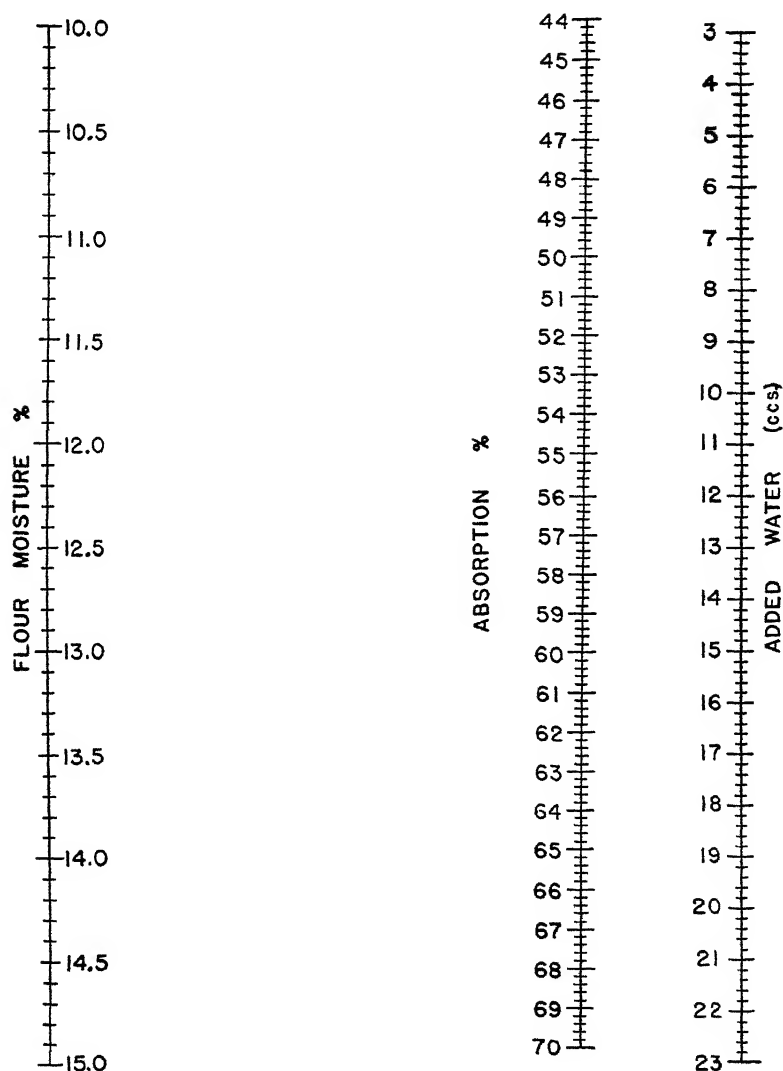


Fig. 1. Nomogram for computing flour absorption to a 13.5% moisture basis, using a flour weight corresponding to 86.5 g. dry matter and the stock solutions specified in the A. A. C. C. baking test.

With the basic baking formula, the nomogram is used as illustrated, but for the malt-phosphate-bromate formula the volume of the stock solutions of these ingredients used per test must be considered as

"added water"; for example, if 14 cc. of distilled water, 1 cc. of malt solution, and 1 cc. of potassium bromate solution were used, the value for "added water" would be 16 cc.

### Construction of a Nomogram for Absorption Calculations on Semolina Water Doughs

The method for experimental macaroni processing as outlined by Fifield (1934) and Binnington and Geddes (1936) calls for a 600-g.

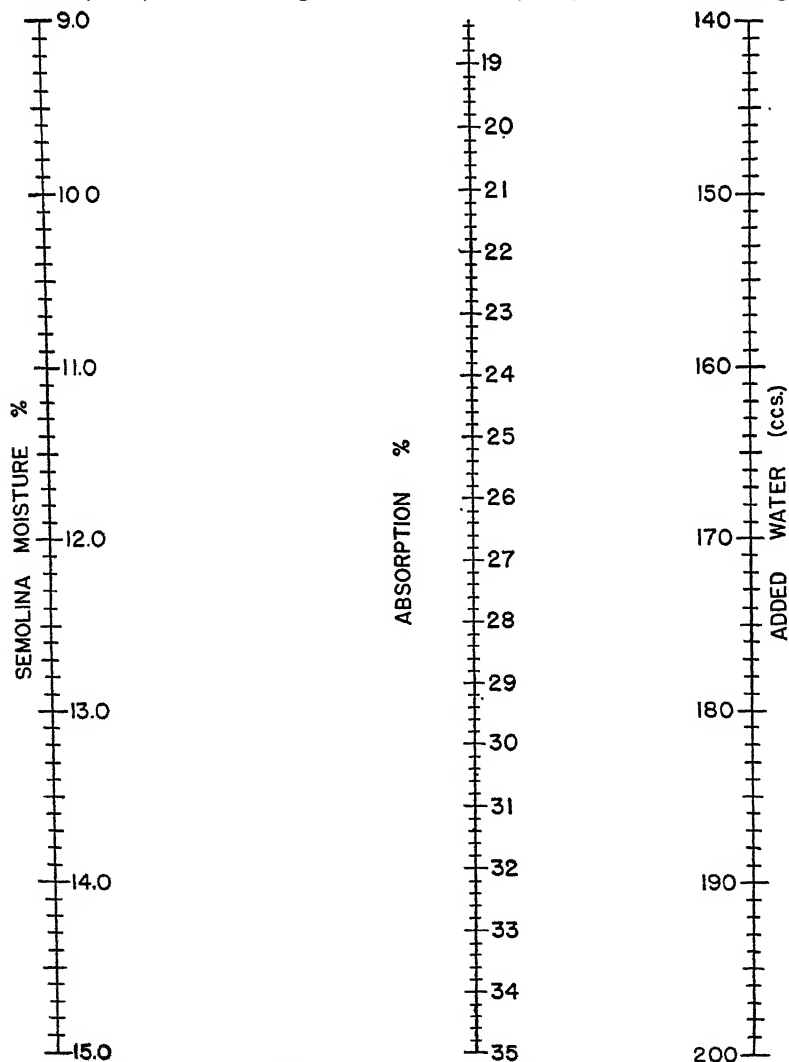


Fig. 2. Nomogram for computing semolina absorption to a 13.5% moisture basis, using a weight of semolina corresponding to 519 g. dry matter (600 g., 13.5% moisture basis).

sample of semolina weighed on a 13.5% moisture basis. Thus the absorption formula becomes:

$$\% \text{ absorption (13.5\% m. b.)} = \frac{\text{cc. added water} - (600 - F)}{600} \times 100,$$

where  $F$  is the weight of semolina required to give 600 g. on a 13.5% moisture basis.

Figure 2 shows a nomogram based on this equation. The scales were constructed in the same manner as the previous example. The moisture range is from 9% to 15% in 0.1% increments, the added water scale reading from 140 to 200 cc. in increments of 1 cc., and the percentage absorption from 19 to 35.

### Summary

Nomograms and their method of construction are given for computing flour and semolina absorptions to a 13.5% moisture basis from experimental baking and macaroni test data. The flour-absorption nomogram is based on a flour weight equivalent to 100 g. on a 13.5% moisture basis and the use of stock solutions as prescribed in the A. A. C. C. baking test procedure. The semolina-absorption nomogram is based on a sample weight of 600 g. (13.5% moisture basis).

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# COMPARATIVE METHODS OF MOISTURE DETERMINATION WITH SPECIAL REFERENCE TO THE BRABENDER GRINDER AND OVEN<sup>1</sup>

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One of the interesting developments of recent years has been the manufacture of new types of laboratory apparatus designed to facilitate the routine testing of cereals and their products. The requirements of the grain trade for rapid and convenient methods of estimating moisture have stimulated the manufacture of various types of electrical moisture meters; several efforts have also been made to simplify conventional laboratory air-oven procedures without sacrifice of accuracy, among which is a combined air oven and balance known as the "semi-automatic moisture tester" by the Brabender Corporation, Germany.

The Brabender oven contains a perforated shelf with spaces for ten moisture dishes; this shelf can be rotated by means of a large knob on the top of the oven so that each dish compartment can be brought directly in front of the small oven door. Underneath the oven, and at the front, is a specially constructed balance which is brought into play by drawing down a lever at the side. One arm of the balance has three long prongs which, when the balance is in operation, project into the oven through perforations on the shelf and lift the dish which is in the front position; the balance is fitted with a damping device and comes to rest when the sample and dish are at equilibrium with the counterpoise on the other arm, the percentage of moisture content being read directly on an illuminated scale.

Since all the dishes have the same tare and a fixed sample weight (10 g.) is employed, the moisture content of each sample can be automatically read in turn by rotating the tray without removing the dishes from the oven. Temperature is controlled by a thermo-regulator, and in the early model forced-air circulation was achieved by means of a ventilating fan which was automatically cut off when the balance scale was illuminated.

As an accessory for determining the moisture content of grain, the Brabender Corporation also developed a new type of laboratory grinder, in which attrition is brought about by means of a small grinding cone oscillated within a ring by an electro-magnetic device. Provision is

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made for adjusting the fineness of grind, and the sample passes directly into a glass container placed close to the orifice in order to reduce moisture loss.

The experiments reported in this paper were undertaken to determine the utility of the Brabender grinder and semi-automatic moisture tester in comparison with equipment commonly used on this continent.

### Experimental

The experiments were designed along the lines of those carried out by Cook, Hopkins, and Geddes (1934, 1935) who undertook a comparative study of different grinders and analytical moisture methods in connection with an investigation of the utility and accuracy of several electrical moisture meters. Three grinders, the Brabender electromagnetic, the Hobart, and the Wiley mill were employed and the moisture content of the ground samples determined in the Brabender semi-automatic moisture tester, DeKhotinsky air oven, and the DeKhotinsky vacuum oven. Moistures were also determined by the Brown-Duvel method and by the two-stage drying procedure outlined by Cook *et al.* (1934, 1935).

Twenty half-gallon samples of hard red spring wheat varying in moisture content from approximately 11% to 20% (Brown-Duvel method) were collected and stored in moisture-tight containers for several weeks, in order to ensure reasonable homogeneity with respect to moisture content. These were subdivided into eight subsamples by means of the one-to-eight progressive riffle described by Cook, Hopkins, and Geddes (1934). The subsamples were placed in half-pint double-tight paint cans and allotted at random to the instruments and methods under test. One subsample was assigned to each grinder and from each grind duplicate moisture determinations were made in each of the three ovens.

In their experiments, Cook, Hopkins, and Geddes (1934) found that a significant loss of moisture occurred during the grinding and exposure of the samples during weighing, etc.; they therefore employed a two-stage drying procedure as a basis of reference and also as a means of securing an estimate of the error involved in subsampling (by running duplicate tests on each of two subsamples). These investigators found that a significant error occurred in subsampling and, accordingly, in the present study two of the remaining subsamples were analyzed by this technique.

The Brabender grinder, which produces a relatively coarse meal, tends, like the Hobart, to heat the sample but to a lesser extent. As it grinds very slowly, it was adjusted largely on the basis of the time required. The Hobart mill was set to grind the high-moisture samples

as finely as possible without choking; in the Wiley mill, a 1-mm. sieve was employed. The times required to grind 100 g. wheat with the settings selected were 215 seconds for the Brabender, 30 seconds for the Hobart, and 90 seconds for the Wiley. As an index of the comparative fineness of grinding, the results of sieving tests are recorded in Table I.

The Brabender moisture tester and the DeKhotinsky (size C) air oven were operated at 130° C. and the samples dried for one hour; for the former a 10 ( $\pm 0.01$ ) g. sample is weighed into a tared aluminium dish (85 mm. in diameter), and for the latter about 2 g. was accurately weighed into tared aluminium moisture dishes provided with inside fitting covers (as specified by the A.A.C.C.), the covers being entirely removed during drying. The vacuum-oven tests of the three grinds were made on approximately 2-g. samples as outlined in Cereal Laboratory Methods (American Association of Cereal Chemists, 1935), except

TABLE I  
RESULTS OF SIEVING TESTS FOR DIFFERENT WHEAT GRINDS

Grinder	U. S. Standard wire sieves				
	Percentage over		Percentage through		
	No. 18	No. 20	No. 40	No. 60	No. 60
	%	%	%	%	%
Brabender electro-magnetic	68.0	10.9	13.1	4.0	4.0
Hobart burr mill	31.9	20.2	35.0	6.6	6.3
Wiley laboratory mill	13.4	23.0	48.0	8.2	7.4

that the drying was continued for 16 hours; the covers of the dishes were loosened but not removed during the drying period, and 20 minutes was allowed for releasing the vacuum. In the DeKhotinsky air- and vacuum-oven tests the dishes were allowed to cool for 20 minutes over calcium carbide before weighing.

Single moisture determinations were made on each of two subsamples by the Brown-Duvel tester operated in accordance with the official methods but with certain additional refinements introduced by this laboratory as outlined by Cook *et al.* (1934).

Two further subsamples were used for the two-stage drying method described by these workers. The first stage consisted of drying  $100.00 \pm 0.01$  g. of the wheat for 72 hours at 72° F. and 40% relative humidity in shallow dishes 4"  $\times$  5", placed in a ventilated cabinet fitted with a screen to exclude dust from the incoming air. At the expiration of this period, all the samples contained about 10.8% moisture and were in approximate equilibrium with the atmosphere. After weighing, the samples were ground in the Hobart burr mill in the same room and duplicate vacuum-oven determinations made on each subsample. The

loss in weight during both stages was then computed in terms of moisture and expressed as a percentage of the original sample.

In addition to the tests on wheat, moisture determinations were conducted on ten samples of flour varying in moisture content from 9.7% to 16.6% (vacuum oven). Duplicate tests were made on 2-g. samples by the official vacuum-oven method and by the 130°C. air-oven method in the Brabender semi-automatic moisture tester and the DeKhotinsky air ovens employing 10-g. and approximately 2-g. samples respectively.

The results are summarized by the statistical constants given in Table II. Appropriate variance analyses were carried out as a measure

TABLE II  
STATISTICAL CONSTANTS FOR MOISTURE DATA ON  
HARD RED SPRING WHEAT SAMPLES

Method	Means of duplicates				
	Brown-Duvel method	Brabender grinder	Hobart burr mill	Wiley mill	All grinders
	%	%	%	%	%
130°C. air oven					
Brabender oven	—	14.80	14.74	14.71	14.75
DeKhotinsky oven	—	14.55	14.51	14.54	14.53
Vacuum oven	—	15.19	15.07	15.07	15.11
All ovens and methods	—	14.85	14.77	14.78	14.80
Brown-Duvel method	14.91	—	—	—	—
Two-stage vacuum oven <sup>1</sup>					
Subsample 1	—	—	15.50	—	—
Subsample 2	—	—	15.47	—	—
Mean differences between duplicates					
130°C. air oven					
Brabender oven	—	0.08	0.13	0.12	0.11
DeKhotinsky oven	—	0.08	0.08	0.09	0.09
Vacuum oven	—	0.05	0.04	0.04	0.04
All ovens and methods	—	0.07	0.08	0.08	0.08
Brown-Duvel method	0.06	—	—	—	—
Two-stage vacuum oven					
Subsample 1	—	—	0.06	—	—
Subsample 2	—	—	0.03	—	—
Standard error of duplicates					
130°C. air oven					
Brabender oven	—	0.08	0.13	0.11	0.11
DeKhotinsky oven	—	0.07	0.07	0.09	0.08
Vacuum oven	—	0.05	0.04	0.04	0.04
All ovens and methods	—	0.07	0.09	0.07	0.08
Brown-Duvel method	0.07	—	—	—	—
Two-stage vacuum oven	—	—	0.04	—	—

<sup>1</sup> Sampling error for two-stage vacuum-oven method is not significant.



of the significance of the differences in the mean values recorded. The difference of 0.03% in the mean moisture content of the two series of subsamples analyzed by the two-stage drying method is not statistically significant, indicating that a negligible error was involved in the subdivision of the samples. It is accordingly valid to compare directly the corresponding moisture values for the different grinders; as determinations were made in the three ovens on each grind, no error in subsampling the whole grain is involved in the oven comparisons for the same grinds. It is noteworthy that, in line with the results of Cook *et al.* (1934), the two-stage vacuum-oven procedure gave higher results than the single-stage vacuum-oven method for the same grind (Hobart), indicating that moisture losses take place in grinding when the moisture in the samples is not at equilibrium with the atmospheric humidity; it seems unlikely that the additional respiration during the preliminary drying could account for the mean difference of 0.42% found.

Considering now the data for the Brabender, Hobart, and Wiley grinds, a much greater variation exists between the various ovens and moisture methods than between grinds. The statistical significance of these variations is shown by the variance analysis given in Table III.

TABLE III  
ANALYSIS OF VARIANCE FOR WHEATS—ALL GRINDERS AND OVENS COMBINED

Variance	D.F.	Variance	F <sup>1</sup>	5% pt. F <sup>2</sup>	5% pt.
Between grinders	2	.2014	—	—	.75 3.23
Between ovens	2	10.0932	—	—	110.19 3.23
Between wheats	19	266.3705	—	—	991.33 1.85
Interactions					
Grinders×ovens	4	.04445	7.04	2.42	— —
Grinders×wheats	38	.2687	42.58	1.45	— —
Ovens×wheats	38	.0916	14.51	1.45	— —
Grinders×ovens×wheats	76	.04418	7.00	1.35	— —
Between duplicates	180	.006311	—	—	— —

<sup>1</sup> Variance "between duplicates" used as error.

<sup>2</sup> Variance due to interactions "grinders × wheats" and "ovens × wheats" were used as errors for testing the significance of differences "between grinders," "between wheats," and "between ovens" respectively.

Because of the high significance of the interactions, these rather than duplicate error were used as sources of error in comparing the differences between grinders and between ovens and moisture methods. This analysis shows that the mean values (three ovens, air- and vacuum-oven methods combined) of 14.85%, 14.77%, and 14.78% for the Brabender, Hobart, and Wiley mills, respectively, are not significantly different. However, individual variance analyses for each oven method show that the Brabender grind gives a significantly higher moisture than the Hobart

and Wiley grinds in the instance of the vacuum-oven procedure. It thus appears that the moisture loss through grinding in the Brabender mill tends to be slightly less than in the Hobart or the Wiley mills; this small difference is of little practical importance. The Hobart and Wiley mills have given virtually identical results in this study although Cook *et al.*, in a series involving 150 comparisons, found that the Hobart mill gave slightly higher moisture values but less consistent results from day to day.

The large and significant variance for "between ovens" is due principally to the much higher mean value of 15.11% for the vacuum-oven method than the 130°C. air-oven values of 14.75% and 14.53% for the Brabender and DeKhotinsky ovens respectively. The Brabender semi-automatic moisture tester operated as a 130°C. air oven gave consistently higher moisture values by approximately 0.2% than the DeKhotinsky oven; this result is perhaps surprising, in view of the fact that a 10-g. sample is used in the former and approximately 2 g. in the latter. However, this difference is apparently counteracted by the greater surface area of the Brabender dishes, the use of forced draft, and the elimination of any possibility of moisture gain during weighing. It is of interest to note that the Brown-Duvel method gave a mean moisture content of 14.91%, which is 0.11% higher than the mean for the Brabender grinder and tester.

The experimental error (standard error of duplicates) is 0.04% for the vacuum-oven methods—a value which agrees closely with that of 0.044% reported by Cook *et al.* (1934). This is appreciably lower than the errors of 0.13% and 0.07% for the Brabender and DeKhotinsky air oven procedures for the same grind. For all grinds combined, the experimental errors of 0.11% and 0.08% for these two air ovens are closely similar. Cook *et al.* (1934) obtained standard errors of 0.07% and 0.05% for the DeKhotinsky air-oven determinations on samples ground in the Hobart and Wiley mills respectively. The standard error of 0.07% for the Brown-Duvel method compares favorably with 0.05% found by the above workers in their study involving 300 wheats.

### Results with Flour

The results of moisture tests conducted in the Brabender and DeKhotinsky ovens operated at 130°C. and in the DeKhotinsky vacuum oven (16 hours' drying) on ten flour samples varying in moisture content from 9.7% to 16.6% (vacuum oven) are summarized in Table IV. The two air-oven procedures give closely similar values but they are significantly lower than those furnished by the vacuum-oven method by approximately 0.1%. As in the case of wheats, the vacuum oven has

TABLE IV  
STATISTICAL CONSTANTS FOR MOISTURE DATA ON WHEAT FLOURS

Method	Means of duplicates	Difference between duplicates	
		Mean	Standard error
	%	%	%
130°C. air oven			
Brabender oven	11.74	0.085	0.068
DeKhotinsky oven	11.78	0.059	0.052
Vacuum oven	11.88	0.024	0.021

the lowest experimental error and the Brabender oven slightly the highest. However, the difference between the Brabender and DeKhotinsky errors is not statistically significant. These data indicate that the Brabender semi-automatic moisture tester and the DeKhotinsky oven are quite satisfactory for the A.O.A.C. rapid air-oven method for flour.

### Discussion

While these experiments indicate that the moisture loss due to grinding is essentially similar for the three grinders, their relative placing in this regard would probably differ under other operating conditions. In fact Cook, Hopkins, and Geddes (1934), in studying the suitability of the Hobart and Wiley mills for grinding wheat for the air-oven method found that the Hobart mill gave slightly higher average moisture values than the Wiley, but the standard errors of prediction of vacuum-oven moisture, and of the duplicates, were lower for the latter, showing it to be superior for this determination. In the Wiley mill, those portions of the sample which will pass the sieve escape further reduction and therefore this mill tends to produce a uniform grind at all times. The type of grind obtainable in the Hobart mill is influenced by the setting employed, the moisture content of the sample, and the wear on the burrs; moreover, if many samples are ground in succession, considerable heating occurs. In the present study, only three samples were ground at one session, whereas Cook *et al.* (1934) ground a much larger number. The Brabender grinder will produce a fairly uniform granulation from time to time but yields a relatively coarse meal and is very slow in operation. The times required for the same type of grain for the Hobart, Wiley, and Brabender mills are in the approximate ratio 1:3:7 respectively. However, the Brabender grinder shows a slightly smaller moisture loss than the other two in the instance of the vacuum-oven procedure.

The Brabender oven technique is much more rapid than the usual

air-oven procedure, since the original weighing of the sample calls for no greater accuracy than  $\pm 0.01$  g. and the final weighing is made without transference of the sample from the oven and attendant cooling in a desiccator. The oven accommodates ten samples at a time and while it gives somewhat higher moisture values with wheat than the DeKhotinsky oven when used for the rapid air-oven method, it yields similar values for flour.

### Summary

The Brabender electro-magnetic grinder and semi-automatic moisture tester have been compared with the Hobart and Wiley grinders and the DeKhotinsky air and vacuum ovens in determining the moisture content of 20 samples of wheat varying in moisture content from approximately 11% to 20% and 10 samples of flour containing from approximately 10% to 17% moisture. Determinations were also made by the Brown-Duvel method and a two-stage vacuum-oven procedure, in which the samples were brought to approximate equilibrium with atmospheric humidity before grinding.

The moisture loss during grinding was essentially the same for the three grinders, although the Brabender device tended to give slightly higher moisture values. It is seven times slower than the Hobart and two and one-half times slower than the Wiley, and gives a relatively coarse grind.

In the wheat tests, the two-stage vacuum-oven procedure gave a mean moisture value 0.4% higher than the single-stage method, which was in turn approximately 0.5% higher than for the A.O.A.C. 130°C. air-oven method. The Brown-Duvel method gave a mean value 0.1% higher than the one-stage vacuum-oven technique. The Brabender semi-automatic moisture tester, operated as a 130°C. air oven, gave higher values than the DeKhotinsky oven by approximately 0.2%. The standard error of duplicate determinations was 0.04% for the two vacuum-oven procedures, which is appreciably lower than the standard errors by all grinds of 0.11% and 0.08% for the Brabender and DeKhotinsky air ovens respectively. The Brabender oven technique is more rapid and convenient.

The Brabender and DeKhotinsky ovens, using the 130°C. air-oven methods, gave similar mean values for moisture in flour, which were 0.1% lower than the vacuum-oven moisture. The standard error of duplicates was 0.02% for the vacuum oven, 0.05% for the DeKhotinsky, and 0.07% for the Brabender.

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**BARLEY AND MALT STUDIES. V. EXPERIMENTAL  
MALTING OF BARLEYS GROWN IN 1937<sup>1</sup>**

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The regional investigations on the malting quality of barleys are given in detail for the five standard varieties grown in 1937. The descriptions and the detailed data on the five standard varieties for the three previous years were given before the association in 1937.<sup>2</sup> The present paper therefore represents a progress report on the malting controls and the malts from the five barley varieties for the fourth year of the investigations.

**Growing Conditions During the Season of 1937**

Climatic conditions were relatively favorable for barley development during the early part of the growing season. The epidemic of stem rust on barley developed early enough at many of the stations, however, to cause severe damage in quality. This was especially true at DeKalb, Ill., Madison Wis., Kanawha, Ia., Lincoln, Neb., and Brookings, S. D. In contrast the barleys from Urbana, Ill., and Waseca, Minn., were better than in the previous season. The barleys from the stations farther west, Bozeman, Mont., Fort Collins and Fort Lewis, Colo. (where the barleys were irrigated), and Davis, Cal., were grown under climatic conditions generally similar to the previous seasons and were not damaged by stem rust.

<sup>1</sup> Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station and other states cooperating in the Barley Improvement Council. The federal WPA has contributed to the research through the University of Wisconsin WPA Natural Science Project. The United States Maltsters Association has helped in the investigations through an Industrial Fellowship Research grant to the University of Wisconsin.

<sup>2</sup> J. G. Dickson, A. D. Dickson, H. L. Shands, and B. A. Burkhart, Barley and malt studies: IV Experimental malting of barleys grown in 1936 and summary data for three years 1934, 1935, and 1936, *Cereal Chem.* 15: 133-168, 1938.

### Study of Malting Controls

Two malting controls were used in the routine malting of the barleys grown in 1937. Wisconsin Barbless (Wisconsin Pedigree 38) barley No. 726, grown at Manitowoc in 1937, was used in all eight series, and Oderbrucker (Wisconsin Pedigree 5-1) barley No. 464, grown at Madison in 1936, was used with six of the series of barleys malted and analyzed. The means for the series of runs in the malting chamber together with the standard deviations and coefficients of variability are given in Table I.

The malting chamber was operated with more uniform temperature and humidity control than in the three previous seasons. Temperature adjustment was also improved in the drying process. In addition better equipment was available for weighing the bulk malts. A combination of these conditions resulted in less variability in 1937 than in previous years. The eight runs of the Wisconsin Barbless control had less variability for moisture content of green and dried malt, recovery of malt from barley, soluble nitrogen in the wort, and diastatic power. The variability in kernel weight, extract, and nitrogen was greater than in 1936 and fluctuated about the average for the previous years. The six runs of the Oderbrucker control showed a greater variability than the Wisconsin Barbless control except for kernel weight, moisture content of green malt, and malt nitrogen. Compared with the previous year there was less variability in kernel weight, total nitrogen, and diastatic power and greater variability in extract and soluble nitrogen in the 1937 series of Oderbrucker controls. The data given in Table I show the need of further improvement in controlling variation in the moisture content of the dried malt, which in turn should further reduce the fluctuations in diastatic power of malts having high diastase. The variation in the total nitrogen content has not been reduced by the use of the Wiley mill in grinding the samples.

The use of the same Oderbrucker control as in 1936 gives an interesting comparison of the average composition of the malts from a barley malted the same year as grown and carried over in storage one year and then malted. The barley was steeped and malted at approximately the same moisture content during the two seasons. It was steeped to an average moisture content of 46.3% in 1936 and 45.9% in 1937. The average moisture content at the end of the germination period was 45.4% in 1936 and 44.7% in 1937. The average moisture content of the dried malts was somewhat lower in 1936, 4.8% in contrast to 5.5% in 1937. Recovery of malt from barley, expressed on the dry basis, was 89.5% in 1936 and 89.0% in 1937. The kernel weight

TABLE I  
VARIABILITY OF MALTING CONTROLS USED IN EACH MALTING SERIES IN THE LARGE UNIT IN 1937-1938

Calculations	Moisture content reached in steep	Moisture content green malt	Moisture content dried malt	Recovery of malt from barley, dry basis	Kernel weight of malt, dry basis	Extract yield of malt, dry basis	Total nitrogen in malt	Soluble nitrogen in wort	Dia-static power of malt
	%	%	%	%	mg.	%	%	%	° L.
<i>8 Series</i>									
Wisconsin Barbliss (Ped. 38)									
Mean.....	45.9	44.5	5.5	89.8	23.0	71.0	2.15	0.573	126.9
Standard deviation.....	0.410	0.517	0.591	0.866	0.524	0.363	0.075	0.011	4.520
5% point.....	0.9	1.2	1.4	2.0	1.2	0.8	0.2	0.3	10.4
Coefficient of variability.....	0.89	1.16	10.72	0.96	2.27	0.51	3.51	1.97	3.56
<i>6 Series</i>									
Oderbrucker (Ped. 5-1)									
Mean.....	45.9	44.7	5.5	89.0	22.7	74.8	2.11	0.823	146.3
Standard deviation.....	0.587	0.517	0.600	0.902	0.366	0.535	0.063	0.032	9.065
5% point.....	1.4	1.2	1.5	2.2	0.9	1.3	0.2	0.1	22.2
Coefficient of variability.....	1.28	1.16	11.00	1.01	1.61	0.71	2.99	3.89	6.19

of the malt was higher in 1936, 23.7 mg. in contrast to 22.7 mg. in 1937. Extract content, expressed on the dry basis, was 1.1% lower in 1936, that is 73.7% in 1936 and 74.8% in 1937. There was no significant difference in total nitrogen content of the two seasons' malts, although the average soluble nitrogen in the wort was appreciably lower in 1936, 0.674% in contrast to 0.823% nitrogen in 1937. The diastatic power of the malts for the two years was the same, 146.3 in 1936 and 146.6 in 1937. The results with this one lot of barley suggest that storage for one year may increase the extract yield slightly and may result in a significant increase in the soluble nitrogen in the wort, at least with the laboratory mashing procedure.

### Study of Malts from the Barleys Grown in 1937

Fourteen stations furnished barley of the five standard varieties grown in 1937. The quality of the barleys from DeKalb, Ill., Madison, Wis., Kanawha, Ia., Brookings, S. D., and Lincoln, Neb., was comparatively poor. Bushel weight and kernel weight were low and the percentage of shriveled kernels high. The barleys from Urbana, Ill., Ames, Ia., and Waseca, Minn., were about average quality. The grain from the western stations was very similar in quality to that of the previous years (Table II).

The barleys were malted approximately the same as in the previous years. The moisture content of the barley after steeping and during the germination period was lowered somewhat in contrast with the previous year except for the Oderbrucker, which was about the same as in 1936. The barleys were steeped to 46% moisture and malted at 46% to 44% moisture content.

As might be expected from the previous discussion of the barleys, there was a wide range in physical and chemical characters in the barleys and malts from the various locations. This makes it advisable to present the data for the individual stations as well as the average for all fourteen stations (Table II).

The yield in bushels per acre varied greatly, depending in large part upon the date and severity of the stem rust attack in the north-central barley area. The yields at Urbana, Illinois, Ames, Iowa, Waseca, Minnesota, and Fargo, North Dakota, were fairly representative of good barley yields in the spring barley area. Stem rust at these stations either was not severe or it developed too late to influence greatly the yield or quality of the grain. In contrast to the barley grown at these stations, that grown at East Lansing, Michigan, DeKalb, Illinois, Madison, Wisconsin, Kanawha, Iowa and Brookings, South Dakota, was very low in acre yield and below average in the general quality of



TABLE II  
PHYSICAL AND CHEMICAL FACTORS OF BARLEYS AND MALTS FOR THE FIVE STANDARD VARIETIES GROWN IN THE REGIONAL SERIES IN 1937

Location and variety	Yield barley Per acre	Bushel wt. dry basis	Kernel wt. dry basis	Total protein N X 6.25	Ash content %	Wt. of hull %	Est. time steeped to reach 46% moist	Moisture content steeped barley	Moisture content green malt	Recovery malt from barley	Bushel wt. dry basis	Kernel wt. dry basis	Moisture content of malt	Moisture content of malt	Extract fine dry basis	Time of conversion	Diastatic power °L	Total nitrogen malt	Total protein malt N X 6.25	Soluble nitrogen in wort	Wort nitrogen malt N	% relation soluble to total N	Formal nitro- gen relation malt N
<i>Oederbrucker (Ped. 5-1)</i>																							
East Lansing, Mich.	20.2	35.4	24.5	11.32	3.05	15.6	41	46.0	45.6	88.9	32.3	22.2	4.9	73.1	7-10	128	1.88	11.76	7.02	37.3	36.2	6.54	
Urbana, Ill.	43.7	41.3	27.5	15.56	15.5	15.5	38	43.2	44.6	89.7	36.6	24.9	4.7	75.1	7	162	2.07	12.89	7.16	34.6	33.7	6.57	
DeKalb, Ill.	16.3	38.9	25.2	13.06	3.15	14.2	30	47.1	44.6	89.5	33.5	24.3	4.7	72.1	5	147	2.16	13.50	6.95	32.2	30.6	5.88	
Madison, Wis.	17.5	28.9	18.0	13.25	3.47	22.2	12	49.5	44.9	88.8	27.2	16.4	4.6	66.5	25	134	2.13	13.32	7.12	33.4	32.8	6.53	
Waseca, Minn.	38.3	41.8	26.1	11.81	3.43	15.2	38	46.4	45.8	87.7	34.9	21.4	5.8	74.5	5-7	168	1.90	11.90	6.93	36.5	35.2	6.63	
Kanawha, Ia.	4.2	36.5	19.6	13.79	3.80	18.5	14	53.0	52.0	84.1	28.0	17.3	5.0	69.4	5	215	2.19	13.67	8.67	39.6	38.4	7.85	
Ames, Ia.	—	36.5	19.2	16.06	3.36	16.0	17	48.4	45.3	88.7	31.1	17.5	4.5	71.3	5	208	2.66	16.62	9.76	36.7	36.0	7.03	
Fargo, N. D.	28.3	37.0	21.0	15.81	3.11	17.4	13	50.4	53.3	83.2	27.0	17.1	5.1	70.3	5	212	2.73	17.06	12.10	44.3	42.1	9.60	
Brookings, S. D.	2.3	34.0	19.2	21.06	3.44	17.8	16	53.4	43.6	92.4	31.4	17.5	6.0	66.9	<5	333	3.56	22.25	10.90	30.6	28.1	4.76	
Lincoln, Neb.	10.3	36.7	17.4	19.12	3.71	19.8	14	46.9	44.1	89.9	33.0	15.5	5.9	65.9	5	252	3.15	19.68	7.65	28.2	26.1	4.76	
Bozeman, Mont.	53.6	47.4	36.1	19.50	2.87	9.6	38	45.6	45.6	88.2	37.5	28.2	5.7	74.4	5	210	2.60	16.25	7.85	30.2	28.4	5.54	
Fort Collins, Colo.	45.0	46.9	30.6	15.37	2.65	9.6	36	46.2	45.6	88.2	37.5	28.2	5.7	74.4	5	210	2.60	16.25	7.85	30.2	28.4	5.54	
Fort Lewis, Colo.	45.0	46.0	31.3	10.94	3.10	10.9	41	46.2	45.3	89.6	39.4	29.0	5.5	76.0	7	142	1.85	11.58	3.67	30.6	28.1	4.37	
Davis, Calif.	11.1	45.0	35.5	12.34	3.00	10.5	68	43.9	43.9	90.2	40.5	31.8	5.5	76.4	7-10	167	2.05	12.83	6.87	33.4	31.2	6.05	
Average	24.40	38.6	25.8	14.93	3.23	15.2	26	47.6	46.0	88.5	33.6	22.3	5.3	71.9	7	193.2	2.40	15.01	8.23	34.4	32.8	6.44	
<i>Wisconsin Barbless (Ped. 58)</i>																							
East Lansing, Mich.	25.1	36.6	27.2	12.40	3.02	13.5	32	47.3	44.4	89.3	33.3	24.7	5.9	71.4	10	125	1.99	12.45	6.38	32.1	31.0	5.98	
Urbana, Ill.	52.1	38.1	25.3	13.25	3.18	16.9	27	46.3	43.8	88.7	32.4	22.8	5.9	70.9	5	124	2.21	13.79	6.59	29.8	28.2	5.57	
DeKalb, Ill.	23.4	36.9	23.0	12.45	3.35	15.9	19	47.0	44.7	89.7	31.3	20.0	4.5	71.4	5-7	146	2.06	12.89	7.33	35.6	33.3	6.84	
Madison, Wis.	26.6	30.2	22.3	11.81	3.17	18.1	16	48.9	43.3	90.8	30.3	20.4	5.3	68.1	20-25	74	1.90	11.90	5.38	28.3	26.7	4.89	
Waseca, Minn.	37.1	43.5	28.6	12.25	3.15	14.1	49	45.8	45.2	88.4	36.6	25.4	5.2	74.1	10	119	1.87	11.70	5.91	31.6	30.3	5.24	
Kanawha, Ia.	31.6	36.0	24.3	13.25	3.19	16.9	36	47.3	44.2	90.0	34.8	21.4	5.1	70.4	10-15	141	2.07	12.95	6.24	30.1	29.5	5.02	
Ames, Ia.	48.1	38.7	24.6	15.25	3.06	14.3	49	45.6	44.7	89.7	34.4	22.7	5.1	70.0	7-10	144	2.53	15.81	7.07	29.1	27.2	4.27	
Fargo, N. D.	48.1	37.9	23.3	13.93	2.84	17.7	28	48.0	45.4	89.7	32.3	21.0	5.4	68.5	10	203	3.19	19.94	8.45	26.3	24.8	—	
Brookings, S. D.	11.2	32.4	18.1	18.86	3.15	20.0	13	48.4	43.9	90.3	29.3	17.0	5.4	63.8	7-10	142	2.35	14.47	5.39	29.9	27.7	—	
Lincoln, Neb.	26.7	47.3	32.2	17.12	3.23	18.9	25	47.3	43.9	89.3	37.9	31.1	6.2	74.2	7-10	145	2.43	15.67	5.39	22.9	21.9	3.32	
Bozeman, Mont.	70.4	47.3	35.3	17.93	2.72	10.8	48	46.2	44.3	88.6	39.5	32.1	5.8	73.0	20	144	2.43	15.19	6.28	25.8	25.1	3.99	
Fort Collins, Colo.	48.6	43.8	28.8	14.61	2.68	10.8	53	46.0	44.4	89.1	36.8	27.0	5.8	73.0	20	144	2.43	15.19	6.28	25.8	25.1	3.99	
Fort Lewis, Colo.	47.0	41.3	28.1	9.63	3.11	11.2	45	46.4	44.7	90.6	38.2	26.0	5.8	73.0	20	144	2.43	15.19	6.28	25.8	25.1	3.99	
Davis, Calif.	37.0	41.3	36.1	11.20	3.00	13.6	66	46.2	43.3	90.6	38.9	33.1	5.3	74.6	10-15	100	1.82	11.38	5.30	29.1	28.3	5.33	
Average	39.09	38.7	26.2	13.85	3.06	15.2	38	46.7	44.3	89.6	34.3	23.8	5.4	70.7	10.5	129.6	2.21	13.85	6.32	28.6	27.4	4.85	
<i>Yabai (Minn. 447)</i>																							
East Lansing, Mich.	27.8	37.1	24.9	11.52	3.07	15.5	32	48.3	44.3	88.7	33.1	21.4	4.7	72.9	7-10	118	1.93	12.08	7.16	37.1	35.6	6.32	
Urbana, Ill.	45.6	41.7	27.4	13.67	2.96	13.5	39	43.4	44.3	89.7	36.2	25.1	5.2	74.7	7	158	2.23	13.93	7.68	34.4	33.2	6.64	

TABLE II—*Continued*

<i>Velut (Miss. 447)—Cont.</i>	bu.	lbs.	mg.	%	hrs.	%	%	lbs.	mg.	%	min.	° L.	%	%	%	%
DeKalb, Ill.	17.0	37.4	23.6	12.83	3.47	15.8	44.1	88.1	30.3	19.4	4.8	70.1	149	2.18	13.62	814
Madison, Wis.	19.5	31.1	25.9	12.51	3.38	16.6	46.6	89.4	29.2	17.5	5.0	68.3	106	1.95	12.19	373
Waseca, Minn.	44.6	43.1	25.1	11.90	3.39	14.3	46.3	88.5	35.6	22.0	5.4	75.6	128	1.83	11.46	690
Kanawha, Ia.	20.3	34.1	21.5	12.89	3.97	18.5	45.3	88.7	33.1	19.5	4.7	70.5	135	2.02	12.63	730
Ames, Ia.	42.0	36.5	20.3	13.59	3.25	17.3	47.2	88.7	32.0	18.1	4.4	69.9	196	2.51	15.69	824
Fargo, N. D.	42.4	37.1	23.3	13.99	3.04	16.0	48.8	88.7	32.1	20.3	5.3	69.7	171	2.35	14.67	797
Brookings, S. D.	10.4	33.8	18.1	16.62	3.30	19.3	45.7	88.7	32.0	17.8	5.0	69.7	171	2.35	14.67	797
Lincoln, Neb.	71.7	36.2	30.4	17.66	3.40	19.3	44.8	88.9	32.6	17.8	5.0	69.7	214	2.02	18.20	1060
Bozeman, Mont.	31.7	36.2	30.4	17.66	2.83	12.0	46.2	88.9	32.6	17.8	5.0	69.7	214	2.02	18.20	1060
Fort Collins, Colo.	24.7	44.8	30.4	16.12	2.91	11.7	37	88.9	38.9	30.7	6.0	73.7	154	2.33	14.55	574
Fort Lewis, Colo.	31.7	44.8	30.4	16.12	2.91	11.7	37	88.9	38.9	30.7	6.0	73.7	154	2.33	14.55	574
Davis, Calif.	16.4	46.0	33.3	12.13	2.91	10.2	53	88.9	38.9	30.7	6.0	73.7	154	2.33	14.55	574
Average	31.16	39.1	25.2	14.05	3.21	15.3	27	88.9	34.0	22.3	5.3	72.0	153.3	2.23	13.76	775
<i>Monacharia (N. D. 2121)</i>																
East Lansing, Mich.	16.6	35.9	24.0	12.40	3.19	15.1	49.4	89.0	31.8	21.9	6.1	74.7	178	2.04	13.06	868
DeKalb, Ill.	14.3	37.2	27.3	13.38	3.17	12.9	32	89.4	35.2	22.7	5.1	75.1	166	2.29	13.99	762
Madison, Wis.	14.3	37.2	27.3	13.38	3.17	12.9	32	89.4	35.2	22.7	5.1	75.1	166	2.29	13.99	762
Waseca, Minn.	14.8	27.9	17.3	13.50	3.50	13.8	18	89.4	30.6	19.5	5.1	71.9	174	2.29	14.31	850
Kanawha, Ia.	47.7	42.0	25.1	13.00	3.33	13.9	34	89.4	35.5	24.6	6.3	75.3	191	2.17	13.56	801
Ames, Ia.	11.3	31.0	20.0	14.05	4.01	14.7	19	89.4	31.3	17.0	4.5	70.4	187	2.28	14.25	842
Fargo, N. D.	34.9	36.7	22.0	16.55	3.34	14.0	23	89.4	32.4	22.6	4.5	71.4	201	2.66	16.62	919
Brookings, S. D.	3.7	33.7	18.7	19.86	3.30	15.9	12	89.4	33.8	18.6	5.4	69.8	184	2.36	14.73	892
Lincoln, Neb.	13.2	35.0	18.3	18.86	2.92	15.9	12	89.4	33.8	18.6	5.4	69.8	342	3.18	19.86	1080
Bozeman, Mont.	99.9	40.7	24.0	13.66	2.92	15.9	45	89.4	33.8	18.6	5.4	69.8	213	2.52	17.06	798
Fort Collins, Colo.	25.0	45.8	28.2	11.76	3.09	10.5	35	89.4	33.8	18.6	5.4	69.8	210	2.56	16.00	834
Fort Lewis, Colo.	15.6	44.5	34.9	11.64	2.91	11.9	57	89.4	33.8	18.6	5.4	69.8	152	1.90	11.90	688
Davis, Calif.	26.68	38.3	24.2	14.59	3.06	14.0	23	88.7	33.2	21.9	5.5	72.5	196.6	2.39	14.94	841
Average																
<i>Trebis</i>																
East Lansing, Mich.	30.6	35.2	34.2	11.14	2.89	15.8	32	90.5	33.9	32.8	5.6	74.5	125	1.86	11.64	663
DeKalb, Ill.	54.3	41.5	26.7	12.33	3.16	14.8	34	90.5	33.9	32.8	5.6	74.5	125	1.86	11.64	663
Madison, Wis.	24.7	37.4	21.5	12.08	3.02	14.0	33	89.4	32.7	28.0	5.2	70.8	143	2.30	14.36	748
Waseca, Minn.	27.7	37.4	21.5	12.08	3.02	14.0	33	89.4	32.7	28.0	5.2	70.8	143	2.30	14.36	748
Kanawha, Ia.	68.1	42.0	35.1	11.46	3.11	14.0	61	89.4	32.7	28.0	5.2	70.8	105	1.99	12.45	552
Ames, Ia.	28.1	36.1	24.1	13.38	3.43	15.1	42	89.4	35.8	31.7	5.7	75.6	168	2.21	13.79	667
Fargo, N. D.	43.5	37.7	29.4	15.81	2.81	14.4	35	89.4	34.2	28.7	5.2	72.4	168	2.21	13.79	667
Brookings, S. D.	47.2	36.0	29.6	14.87	2.95	15.6	29	89.5	33.8	28.7	5.4	68.4	187	2.19	13.67	728
Lincoln, Neb.	17.0	31.7	24.1	17.74	3.19	19.7	19	89.5	32.2	26.5	5.4	68.4	168	2.19	13.67	728
Bozeman, Mont.	23.2	35.8	26.5	17.43	3.08	16.5	28	89.5	32.8	24.1	6.3	69.1	271	2.91	18.18	864
Fort Collins, Colo.	91.4	46.3	34.4	14.55	2.73	14.1	51	89.5	32.8	24.1	6.3	69.1	216	2.51	17.74	774
Fort Lewis, Colo.	55.1	40.3	29.5	13.57	2.96	13.1	64	89.5	32.8	24.1	6.3	69.1	160	2.35	14.67	798
Davis, Calif.	26.1	44.2	39.2	10.64	2.87	13.8	73	89.5	32.8	24.1	6.3	69.1	160	2.35	14.67	798
Average	44.33	38.9	33.8	13.51	2.99	14.7	43	90.5	35.5	30.8	5.7	72.7	149.8	1.98	13.68	638

the grain. The barley yields at East Lansing, DeKalb, Kanawha, and Brookings were lower than in any of the previous years and the yields were slightly higher at Madison than in the two dry years, 1934 and 1936.

The general quality of the barleys showed a variation similar to that in yield at the different stations. The bushel weight and kernel weight of the barleys were usually low at the stations where stem rust was severe and about average at other stations. The barleys probably were more severely affected by rust at Madison, Wisconsin, than at any other station. The stem rust infection at Madison reduced bushel weight and kernel weight but did not reduce yield in bushels per acre below those of the unfavorable seasons of 1934 and 1936. In other words, the stem-rust epidemic seemed to affect bushel and kernel weight more than it did yield per acre.

The extract content of the malts showed the influence of the stem-rust epidemic in certain localities although the effect was not so clearly defined as in certain other quality factors. Extract content of the malts from all varieties was low at Madison, Wisconsin. The average extract for the five varieties at this station in 1937 was 68.1%, in contrast with 71.6% in the dry year 1936, 76.2% in 1935, and 70.2% in 1934. Extract content was low for the malts from Kanawha and Ames, Iowa, Brookings, South Dakota, Fargo, North Dakota, and Lincoln, Nebraska. The extract content of the malts from the other stations was about average except that those from Waseca, Minnesota, East Lansing, Michigan, and Bozeman, Montana, averaged higher than in the three previous years.

The nitrogen content of the barleys and malts was in general lower than in the previous year for most of the barleys grown in the main malting barley area and California. The two more southern stations, Urbana and DeKalb, Illinois, and the more western stations in the spring barley area (*i.e.*, Ames, Iowa, Brookings, South Dakota, Lincoln, Nebraska and Bozeman, Montana) produced barleys which were higher in nitrogen, in many instances, than in either 1935 or 1936.

Diastatic power of the malts showed a wide variation between stations and in general showed a distribution somewhat comparable to total nitrogen content of barleys and malts. This is shown also by the high correlation between nitrogen and diastatic power for the 1937 season,  $r = +.8743$ . The very high diastatic powers of the malts from Brookings, South Dakota (Table II), were rather unusual and worthy of special mention. The malt from the Manchuria had a diastatic power of 342, Oderbrucker 333, Velvet 224, Trebi 216, and Wisconsin Barbless 203° L. Diastatic values from Fargo, North Dakota, were also higher than usual. The malts from Madison,

Wisconsin, were low in diastatic power, averaging 112° L. for the five varieties in contrast with 130° L. in 1936 and 158° L. in 1935. The diastatic values on malts from East Lansing and Waseca were lower than usual.

The other factors determined on the barleys and malts showed a similar wide range between the different stations and years. For example the rate of water absorption was faster with the 1937 barleys, and hull content was much higher than in the previous years. Space will not permit a discussion of all of these in detail. The data are given in Table II and can be compared with the previous years by referring to the tables given in Barley and Malt Studies IV.<sup>3</sup>

### The Average for the Fourteen Stations Considered

The averages of the data from all fourteen stations show the relative quality of the barley varieties for the season of 1937, but these averages are not entirely comparable with those of previous years because the number of stations supplying samples varied from year to year (Table II). The averages for 1937 compared with those for all stations in previous years show that in 1937 the barleys were relatively low in bushel weight and had a slightly reduced kernel weight and a fairly mellow endosperm. The protein content was about the same as in 1935 and 1936, but not so high as in 1934. The extract content of the Velvet, Oderbrucker, and Manchuria malts was lower than in the two previous years, while that of Wisconsin Barbless and Trebi was slightly higher than in 1936 and appreciably higher than in 1934. The diastatic power of the malts was approximately the same as in 1936 except for the Wisconsin Barbless, which was significantly higher. In general, averages indicated enough stem-rust damage at several of the stations to decrease the kernel size and the extract without making a typically hard barley. This was also suggested in the reduced time required for water absorption.

### Six Stations Considered

The averages for six stations for each of the four years gives a direct comparison of the influence of seasonal conditions and the stem-rust epidemic upon the quality of the barleys and malts of the five standard varieties. The six stations included in the average are: East Lansing, Mich., Madison, Wis., Waseca, Minn., Kanawha, Ia., Brookings, S. D., and Bozeman, Mont. The varietal averages for four years (1934-1937) and the averages for the five varieties combined for each year are given in Table III. Yields in bushels per acre of

<sup>3</sup> See footnote 2.

TABLE III

AVERAGES FOR THE FIVE STANDARD VARIETIES FOR THE FOUR YEARS, 1934-1937, AND YEARLY AVERAGES FOR THE FIVE VARIETIES COMBINED FOR THE SIX STATIONS: EAST LANSING, MICH., MADISON, WIS., WASECA, MINN., KANAWHA, IA., BROOKINGS, S. D., AND BOZEMAN, MONT.

Factors	Varietal average for 4 years (1934-1937)					Yearly average for 5 varieties			
	Oder-brucker	Wisconsin Barbliss	Velvet	Man-churia	Trebi	1934	1935	1936	1937
Acre yield barley (bu.)	28.1	40.9	37.0	31.1	47.0	32.9	45.1	36.9	31.4
Bushel weight barley, dry basis (lbs.)	38.8	39.7	40.1	39.4	39.0	39.4	41.1	40.0	37.8
Bushel weight malt, 4% basis (lbs.)	35.9	36.5	36.1	35.2	35.6	36.1	37.9	35.3	34.2
Kernel weight barley, dry basis (mgs.)	25.7	27.3	25.3	24.6	33.7	29.8	27.0	26.3	26.1
Kernel weight malt, dry basis (mgs.)	22.8	24.5	22.4	21.7	30.5	27.1	23.5	23.5	23.5
Hull content barley, dry basis (%)	13.4	13.4	13.5	12.2	13.1	12.5	10.9	13.3	15.8
Recovery malt from barley, dry basis (%)	87.0	89.0	88.2	87.0	89.6	87.8	86.4	89.2	89.2
Extract content, fine grind, dry basis (%)	72.0	69.9	71.4	72.5	72.2	71.6	72.7	70.8	71.2
Total protein in malt (%)	15.6	14.6	14.8	15.2	14.0	16.5	14.3	14.3	14.2
Soluble nitrogen in wort as protein (%)	5.3	4.0	4.9	5.5	4.1	5.0	5.0	4.6	4.6
Soluble nitrogen in relation to malt N (%)	34.4	27.4	34.4	35.6	29.2	29.7	35.0	31.6	32.6
Conversion time (min.)	5.6	8.7	5.8	5.8	10.8	7.7	5.9	6.2	9.6
Diastatic power (°L.)	185	116	142	180	138	109	164	168	168

barley averaged low in 1937 and approximately the same as in 1934, which was a very dry year at five of the six stations. The varieties remained in the same order in yield during the four years with the exception that Wisconsin Barbless yielded higher than Trebi in 1935. The order of varieties from high yield to low was as follows: Trebi, Wisconsin Barbless, Velvet, Manchuria, and Oderbrucker. The average kernel weight of barley was lowest in 1937 for all varieties except Trebi. Average extract content was lowest in 1937 for the three varieties, Oderbrucker, Manchuria, and Velvet, and was higher for Wisconsin Barbless than in 1934 and 1936. Trebi was higher in average extract yield than in 1936. The average protein content of barleys and malts was relatively similar during the past three years. Likewise the average diastatic power for the malts of the five varieties was similar to the averages of previous years. The malts from the five varieties held the same relative order for diastatic power for the four years with the exception that Trebi malts were lower than the Velvet malts in 1934 and 1935. In general the malts from the six stations in 1937 were about the same quality as those of 1936 although seasonal conditions during the growing period were decidedly different in the two years. The stem-rust epidemic in 1937 seemed extensive and severe enough to reduce the average quality of the malts from the six stations as much as the continued hot, dry weather of 1936.

### Analysis of Variance

The data from the six stations where all five varieties were grown in four consecutive years were subjected to the analysis of variance. The results in terms of *F* values for some of the factors are presented in Table IV. In general the analysis shows the same type of reactions and interactions as reported on the three years data before this society a year ago. Briefly summarized, the varieties show significant differences for all factors except bushel weight of malt. The stations where the barleys were grown and years in which barleys were grown showed significant differences for all factors studied. The varieties held the same relative ranking at the six different stations for all of the factors except yield of grain and also held the same relative ranking for each of the four years for all factors except bushel weight of barley, protein content of malt, soluble nitrogen in wort, and diastatic power. And finally as might be expected, the stations interacted significantly with years, indicating that stations responded differently for all factors studied during the four seasons. The results suggest that factors making up quality are relatively stable within these five varieties and are not subject to differential responses caused by season or location, with the exceptions mentioned above.



## Correlations

Correlations between certain factors of barley and malt for the individual years and for the four years combined are given in Table V. The negative correlation between malt protein and extract is significant in 1934 and 1937 and in the combined data for the four years. There is a significant positive correlation of malt protein with diastatic power of malt and with soluble nitrogen in the worts for each individual year and for the combined four years. Low kernel weight of the barleys is correlated with high diastase and soluble nitrogen, and low extract.

TABLE V

CORRELATIONS BETWEEN CERTAIN FACTORS OF BARLEY AND MALT FROM THE STANDARD REGIONAL VARIETY SERIES FOR INDIVIDUAL YEARS AND FOR THE FOUR YEARS

Factors correlated	Correlation values for years and four years				
	1934	1935	1936	1937	Four
Protein, malt-extract, malt	-.6733	-.2828	-.1447	-.4730	-.3165
Protein, malt-diastase, malt	+.6538	+.4420	+.6363	+.8743	+.3480
Protein, malt-soluble N, wort ..	+.7950	+.5167	+.6382	+.7209	+.5476
Kernel wt., barley-extract, malt ..	+.2514	+.5955	+.4835	+.5172	+.5796
Kernel wt., barley-protein, barley..	-.4842	-.4092	-.2346	-.1900	-.1296
Kernel wt., barley-diastase, malt.	-.4831	-.6658	-.0818	-.2085	-.3488
Kernel wt., barley-soluble N, wort.	-.6817	-.7930	-.6467	-.5906	-.6031
Moisture content, malt-diastase malt . . . . .	-.0702	+.0664	+.4000	+.4656	+.4150
Moisture content, malt-soluble N, wort . . . . .	-.0115	-.0462	-.2867	-.1500	-.0634
Diastase, malt-conversion time in mashing. . . . .	-.6200	-.3810	-.3870	-.5546	-.4265
5% point . . . . .	.349	.349	.349	.349	.170
1% point. . . . .	.449	.449	.449	.449	.230

The relation of kernel weight to barley protein is not so consistent. The barleys for the season of 1935, the year when all barleys apparently reached full development of the kernels, show significant correlations between kernel weight and a number of other factors. This suggests that kernel weight is of more importance in years when barley reaches full development, than in less favorable years. There is a significant negative correlation between diastatic power of the malts and conversion time in mashing.

The significant positive correlation between moisture content of the malt at the end of the drying period and diastatic power of the



malt is important, especially from the standpoint of methods of malting. The correlation values for the first two years are not significant, while those for the latter two years are significant. Sufficient data are not available to explain this difference in relationship between moisture and diastatic power. The relation between moisture content of the malt and diastatic power is known to maltsters and malting chemists as is shown by the differences in moisture content of brewers' and distillers' malts. Numerous other correlations concerning barley and malt characteristics have been calculated but will be reserved for later papers.

In summation, the malts from the barleys produced in 1937 at approximately one-half of the stations approached the quality of the malts from barleys grown in the two other unfavorable years of 1934 and 1936. In certain quality characteristics the barleys and malts differed from those of the two unfavorable years.

### Summary

Two control barleys were used with those malted and analyzed in the study of the 1937 samples. Variability in certain factors in the controls was reduced somewhat below that of the previous years, in certain other factors it remained about the same, while in still others it increased.

The barleys grown in 1937 at most of the stations in the north-central area were reduced in quality somewhat by the epidemic of stem rust. These barleys were low in yield and bushel and kernel weight and high in hull content. The barleys had about average protein and relatively high ash, approaching that of 1935. The endosperm however was mellow and rate of water absorption rather rapid.

The malts produced from the five standard varieties were somewhat low in extract, slightly low in soluble nitrogen, and about average in protein and diastatic power.

The statistical study of the four years' data for the five standard varieties grown at six stations shows that the varieties, stations where grown, and seasons in which they were grown all had a significant influence on most of the factors studied. The analysis indicates that the five varieties used in the investigations remained in essentially the same ranking at each of the stations where they were grown and in each year grown. However, stations responded differentially in certain years.

Correlations are given for a few of the factors determined on the barleys and malts for the individual years and for the total of the four years. The correlations suggest a different relationship between certain factors in the several years.

# APPLICATION OF THE ALUMINUM-PLATE MOISTURE METHOD IN THE MALTING LABORATORY

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Much work has already been done on methods for the determination of moisture in cereals and cereal products with an ever increasing trend toward methods which are accurate, convenient, and at the same time more rapid than the official methods recognized. Out of these researches have come the facts which are the basis for the more rapid air-oven moisture tests. Davis (1935) showed that by feeding the drying oven with forced draft by means of a vacuum pump attached to the top of the drying-chamber outlet, and raising his temperature to 140°C. for 20 minutes, results could be obtained with wheat flour which were comparable to those by the one-hour method at 130°C. Sandstedt (1938) obtained particularly pleasing results by drying his flour samples on an half-inch aluminum plate for 20 minutes at 140°C. From this work, as also from Anderson (1936), who reduced the time necessary for his samples to come to the temperature of the vacuum oven (100°) from 1½ hours to 10 minutes, by circulating steam through the hollow brass plate on which the samples rested, comes the indication that the problem in rapid drying lies not so much in the removal of vapor accumulating over the drying samples as in the transfer of the required heat to the said samples.

Raising the heating temperature, with corresponding decrease of time in drying, might be another means to the required end, but Davis (1935) and also Treloar and Sullivan (1935) have shown that drying materials such as flour or feed at excessively high temperatures will yield high results because of the decomposition and destructive changes of organic materials present. This latter point is of particularly greater importance in the testing of malt because of the presence of the simpler forms of carbohydrates and proteins and interaction products of these, made available during the process of malting.

With all these facts in view and at the same time considering the fact that work has chiefly been confined to the cereals concerned in the foods and milling industries, it appeared very desirable to apply this line of research to the determination of moisture in the malting control laboratory. It was decided to determine whether or not the aluminum-plate method as recommended by Sandstedt (1938) with certain necessary modifications of time and temperature could be used.

### Experimental

Since the malting laboratory is concerned not only with determinations of the raw grain samples, but also with two types of malt, the tests were run as two independent series, one with the grain and one with the malt, to arrive at optimum conditions. These conditions would necessarily have to yield results comparable to those derived from the methods accepted as standard or official.

One series of tests entailed raw barley samples ground to a floury consistency, and the method of comparison for moisture taken was one recommended by the A. A. C. C. (1935)—namely the air-oven method, 130° for one hour. The other series concerned the processed malt, ground in a Miag-Seck mill, to specifications of the A. S. B. C. (1935) *i.e.*, to yield an approximately 90% grind through a No. 30 U. S. standard sieve. The method of comparison was the official moisture method of the A. S. B. C.—drying approximately 5 grams at 103–5° for three hours.

In this latter series we have brewer's malt, manufactured with a moisture content of approximately 4%, and distiller's malt, which contains about 6% moisture. Because of the comparatively wide range in moisture content between these two types it was decided to run samples in triplicate of each, in separate groups, by both standard and experimental methods. The comparative averages for each sample are given in Table II.

While all the tests were run in the regular Cenco De Khotinsky (new model), the experimental methods were conducted with a solid half-inch aluminum plate used as a tray in the oven for the moisture dishes to sit on, in place of the regular grid shelf. The oven temperatures (read with the bulb buried in a tin of sand, on the aluminum plate) were adjusted to 125°C. for malt and 140°C. for the barley tests.

An half-inch aluminum plate held at room temperature was used to cool the samples dried on the plates in the oven as recommended by Sandstedt (1938). Desiccators were used, however, for all samples heated according to the standard methods. The moisture tins cooled to room temperatures quite comfortably within five minutes. Should longer time be necessary Sandstedt (1938) advises completion of cooling in the desiccator.

The moisture dishes used in both the standard methods for malt and barley and in the corresponding short aluminum-plate method were 55 mm. in diameter and 15 mm. deep. Since approximately five-gram samples are recommended in the standard malt analysis, the same amount was used in the experimental method, while two-gram samples sufficed in all the barley tests to conform with the quantity specified by the A. A. C. C. (1935).

## Results

In Table I are shown the results of a test with malt which confirm data shown by Sandstedt (1938), in the elimination of variations in results due to position in the regular air-oven method on the grid shelf, by using the aluminum-plate method. These results are similar to those of several tests run.

While the variations in the regular air-oven method were rather small, there does appear a tendency toward a slightly higher reading at the back of the oven, except for the left rear corner, with lower readings at the front. This tendency, though small, was noted in all such tests run. On the aluminum plate, however, in the experimental method, it apparently is overcome and results appear uniform, irrespective of position in the oven.

TABLE I  
EFFECT OF POSITION IN OVEN USING STANDARD METHOD AND THE ALUMINUM-PLATE METHOD

Position in oven	Moisture by standard method	Moisture— 125° for 15 min.
	%	%
Left rear corner	4.04	4.10
Center rear side	4.07	4.12
Right rear corner	4.13	4.09
Left center side	4.06	4.16
Center	3.98	4.03
Right center side	4.04	4.04
Left front corner	4.02	4.03
Center front side	4.00	4.13
Right front corner	3.97	4.10

Having confirmed the unimportance of position on the aluminum plate, we placed all replicate samples in subsequent tests on the shelf at random.

Table II shows that while heating for 15 minutes at 125°C. appears to give slightly higher results than the standard method in the majority of cases, decreasing the time to 10 minutes made the drying period too short, particularly for samples with higher moisture, such as distiller's malt.

It is well to note here that the time periods indicated in the experimental methods were considered from the time the oven temperature had risen back to that set, after the oven had been loaded. This was found to yield more uniform and consistent results and overcame the varying lengths of time taken by the oven in returning to required

TABLE II  
AVERAGE MOISTURE DETERMINATIONS ON MALT BY STANDARD METHODS AND BY  
ALUMINUM-PLATE METHOD AT 125°

Sample No.	Standard method	Aluminum-plate method	
		125°, 15 min.	125°, 10 min.
Brewer's Malt	1	4.29	—
	2	3.71	3.66
	3	4.16	4.04
	4	3.77	3.66
	5	3.99	3.91
	6	3.79	—
Distiller's Malt	1	5.91	5.75
	2	5.96	—
	3	6.03	5.90
	4	5.90	—
	5	6.18	6.01
	6	6.21	6.03

temperature, depending on the time its door was open while the samples were put in.

Tests made with the raw grain (only barley was here considered) compare very favorably with results shown by Sandstedt (1938) for ground wheat. In Table III are shown the comparative averages of three replicates for each of six different barley samples by the standard method for cereals (A. A. C. C. 1935) (drying for one hour at 130°C.) and by the short method, at 140°C. for 20 minutes.

TABLE III  
COMPARATIVE RESULTS OF AVERAGES OF BARLEY SAMPLES, RUN BY STANDARD  
METHOD AND BY ALUMINUM-PLATE METHOD—140° FOR 20 MINUTES

Sample No.	Moisture, 130° for 1 hour	Moisture, 140° for 20 min.
	%	%
1	11.18	11.28
2	13.58	13.57
3	14.13	14.12
4	12.67	12.60
5	11.62	12.60
6	11.20	11.20

### Summary and Conclusions

The short aluminum-plate method promises to be of some use in the malting control laboratory. While it requires a little care and caution in the adjustment of time and temperature, and may lack some of the efficiency of the longer standard method, its time-saving character makes it worthy of consideration.

This plate method, as used with the raw cereal, can apparently be used satisfactorily, with considerable degree of accuracy.

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## DOUGH OXIDATION AND MIXING STUDIES

### I. THE ACTION OF POTASSIUM BROMATE IN DOUGH

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(Read at the Annual Meeting, May 1938)

Bread improvers are used to produce desirable improvements in dough. However, good results are not always obtained; under certain conditions the effects of improvers may actually be detrimental. While studying the effects of various bread improvers, we have found that undesirable results in straight doughs might be overcome by remixing them after fermentation.

In a study of the factors concerned in producing these results it was found that the oxidizing agent in the bread improver was responsible for the undesirable results obtained in straight doughs as well as for the great improvement produced by remixing such doughs after fermentation. In the data given below, we shall present a study of the factors involved in the action of potassium bromate in doughs. The factors concerned in the effects of remixing after fermentation will be considered in Section II of this series.

#### The "Excess Bromate" Effect

Jørgensen has studied the effect of oxidizing agents, such as potassium bromate, on doughs. Similar studies, covering a period of years, have been conducted at the Fleischmann Laboratories.

The action of potassium bromate in dough may be illustrated by its effects in the presence of added papain, a powerful proteolytic enzyme. The volume figures in Table I and Figure 1 show that the

TABLE I  
EFFECT OF  $\text{KBrO}_3$  ON THE ACTIVITY OF ADDED PAPAIN IN DOUGH

	Loaf volume
	cc.
Control (no papain, no bromate) . . . .	2050
Papain 75 mg., bromate none . . . . .	1370
Papain 75 mg., bromate 5 mg. . . . .	1380
Papain 75 mg., bromate 10 mg. . . . .	1380
Papain 75 mg., bromate 15 mg. . . . .	1450
Papain 75 mg., bromate 20 mg. . . . .	1520
Papain 75 mg., bromate 25 mg. . . . .	1630
Papain 75 mg., bromate 30 mg. . . . .	1840
Papain 75 mg., bromate 40 mg. . . . .	1920
Papain 75 mg., bromate 50 mg. . . . .	2020
Papain 75 mg., bromate 60 mg. . . . .	2000
Papain 75 mg., bromate 70 mg. . . . .	1990
Papain 75 mg., bromate 80 mg. . . . .	1990
Papain 75 mg., bromate 100 mg. . . . .	1920

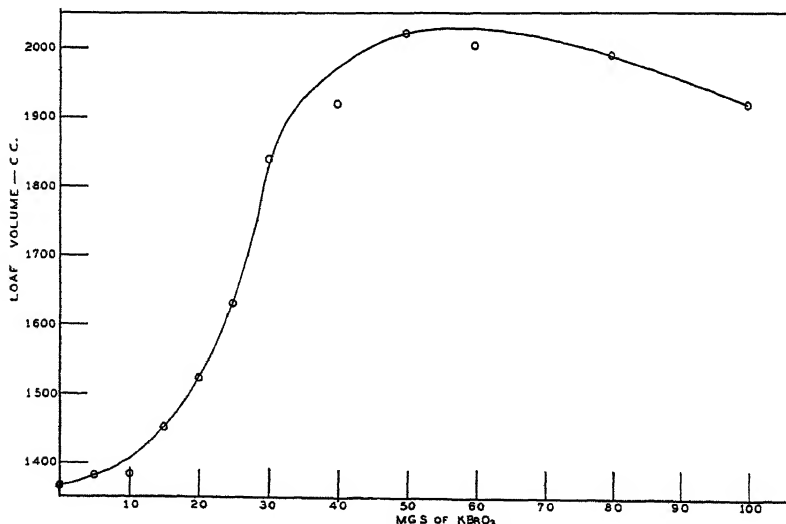


Fig. 1. Effects of increasing amounts of bromate in doughs containing 75 mg. of papain.

proteolytic effects of papain were largely overcome by additions of bromate to the dough; this was also apparent from the condition of the doughs and the appearance of the baked bread.

It will be observed that the volume produced by 100 mg. of bromate is distinctly lower than the optimum; the loaf showed evidence of

what we have designated as the "excess bromate" effect. We have been familiar with this reaction for a number of years, but it has been difficult to develop a satisfactory explanation. Figure 2 shows photo-

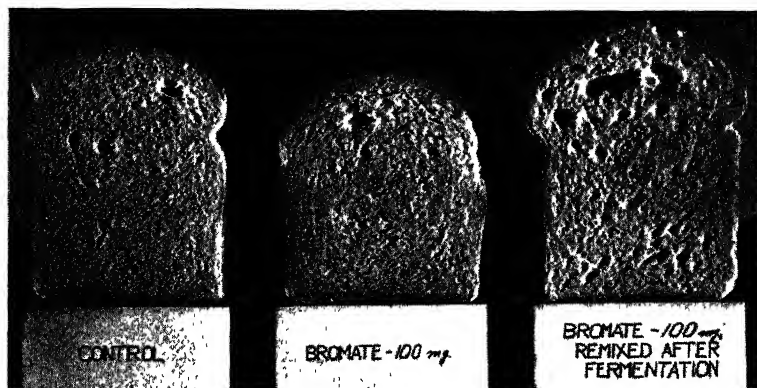


Fig. 2. The "excess bromate" effect, and its elimination by remixing after fermentation

graphs of a no-bromate control, a loaf with 100 mg. of bromate showing a very strong "excess bromate" effect, and a loaf with 100 mg. of bromate made from a dough which was remixed after fermentation. The "excess bromate" effect is characterized by what bakers sometimes refer to as a "bucky" dough, and a loaf of poor volume, rounded corners, rough exterior, a tendency to form a peak on the upper part of the loaf, and poor, heavy, lumpy, coarse texture. We have been particularly interested in this effect, because it is quite common in lean, straight-dough formulas, and goes beyond any point at which it might be explained as due to retardation of proteolytic activity.

Table II and Figure 3 show the effects of excessive amounts of bromate on loaf volume.

TABLE II

EFFECTS OF INCREASING AMOUNTS OF  $\text{KBrO}_3$  ON LOAF VOLUME  
Dough time, 2 hours; pan proof time, 50 min.; pH of bread, 5.48

		Loaf volume
		cc.
Control (no bromate).	...	2140
Bromate 4 mg..	...	2130
Bromate 10 mg..	...	2020
Bromate 20 mg..	...	1900
Bromate 50 mg..	...	1790
Bromate 100 mg..	...	1710
Bromate 500 mg..	...	1610
Bromate 1000 mg..	...	1570



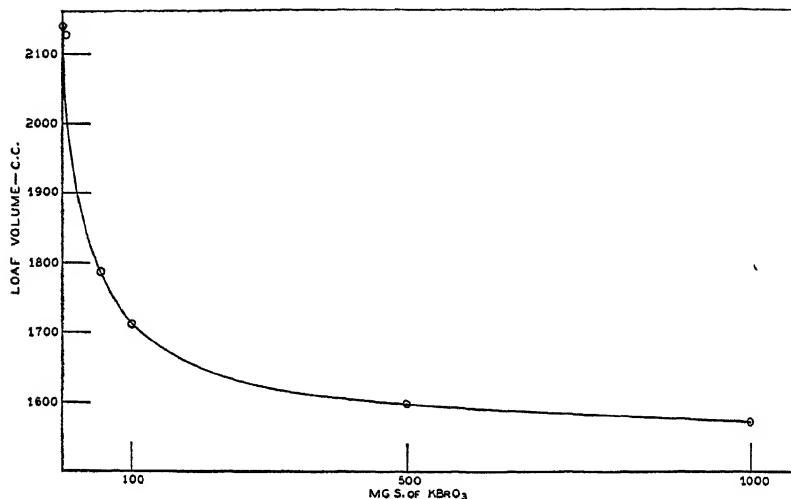


Fig. 3. Effect of bromate on loaf volume.

There is a very sharp decrease in loaf volume, accompanied by increasingly marked "excess bromate" effects, up to about 100 mg., beyond which further increases in the amount of bromate in the dough produce comparatively slight changes in the volume and character of the resulting bread. Qualitative tests indicated a slight amount of undecomposed bromate in the 100-mg. loaf and large amounts in the 500- and 1000-mg. loaves.

The amount of acid developed in the dough as a result of fermentation was possibly a factor limiting the amount of bromate decomposed. This conclusion may be drawn from the results shown in Table III and Figure 4, where the fermentation was prolonged to produce greater acidity in the dough.

Figure 4 shows that 20 mg. of bromate, which is usually considered a large excess, actually produced an increase in volume in the very early stages of fermentation. With increasing fermentation time and the resulting increase in acidity as shown by the pH changes in Table III, there is a progressive decrease in loaf volume to a value much lower than that shown for 20 mg. of bromate in Table II. After three hours of fermentation the change in volume is slight in comparison to the change in pH.

Similar tests with 20, 50, 75, 100, and 200 mg. of bromate were made with another lot of the same kind of flour at a later date. The loaf volume figures are given in Table IV and plotted in Figure 5.

TABLE III

EFFECTS OF INCREASING FERMENTATION TIME ON LOAF VOLUME AND pH, FOR DOUGHS WITH 20 MG. OF  $\text{KBrO}_3$ 

Dough time	Sugar	Pan proof	Loaf volume	pH of bread
<i>Hrs.</i>	<i>%</i>	<i>min.</i>	<i>cc.</i>	
None	5	87	2060	5.70
$\frac{1}{4}$	5	76	2110	5.70
$\frac{1}{2}$	5	73	2190	5.70
1	5	61	2110	5.70
$1\frac{1}{2}$	5	55	2010	5.67
2	5	59	1880	5.62
3	5	50	1770	5.49
4	6	50	1740	5.41
5	7	59	1730	5.29
6	8	65	1680	5.26

In texture, the 0,  $\frac{1}{4}$ , and  $\frac{1}{2}$ -hour loaves were immature; the 1-hour loaf was nearly normal; the  $1\frac{1}{2}$ -hour loaf showed a distinct "excess bromate" effect; and the 2, 3, 4, 5, and 6-hour loaves showed progressively greater "excess bromate" effects, with the 4, 5, and 6-hour loaves quite similar to each other in that respect.

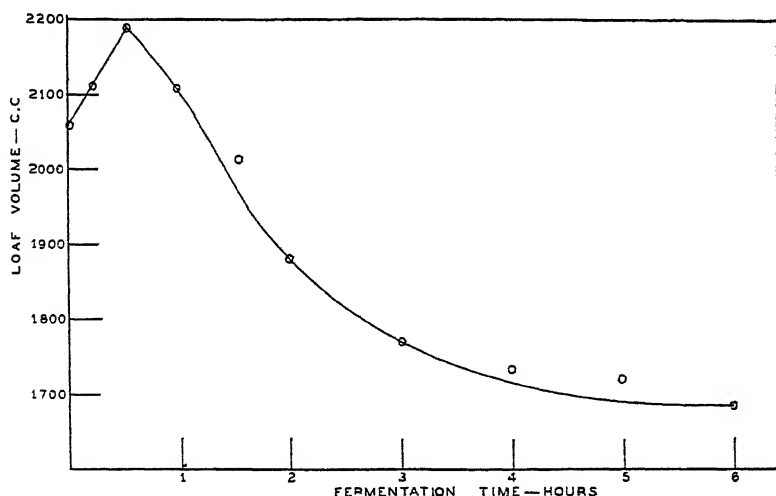


Fig. 4. Effect of fermentation time on loaf volume in doughs containing 20 mg. of bromate.

From a study of the curves shown in Figure 5 the following conclusions seem justified:

1. Excessive amounts of bromate produce great decreases in loaf volume with increasing fermentation time.
2. Loaf volume also decreases as the amount of bromate is increased; this effect seems to be independent of the amount of fermentation and indicates that bromate may produce changes in dough even in the absence of fermentation.

TABLE IV  
EFFECTS OF INCREASING FERMENTATION TIME ON LOAF VOLUME WITH VARYING AMOUNTS OF  $\text{KBrO}_3$ <sup>1</sup>

Dough time	Loaf volumes with specified mg. of $\text{KBrO}_3$				
	20	50	75	100	200
Hrs.	cc.	cc.	cc.	cc.	cc.
0	1930	1990	1930	1870	1720
$\frac{1}{4}$	—	2020	—	1900	1670
$\frac{1}{2}$	2070	2020	1930	1680	1620
1	2050	1760	1710	1640	1570
$1\frac{1}{2}$	—	1720	—	—	1540
2	1770	1650	1630	1570	1510
3	1640	—	1550	1500	—
4	—	1550	—	—	1490
5	—	—	—	—	1470
6	1530	1520	—	—	1470

<sup>1</sup> Differences in percentage of sugar, pan proof time, and pH were similar to those shown in Table III.

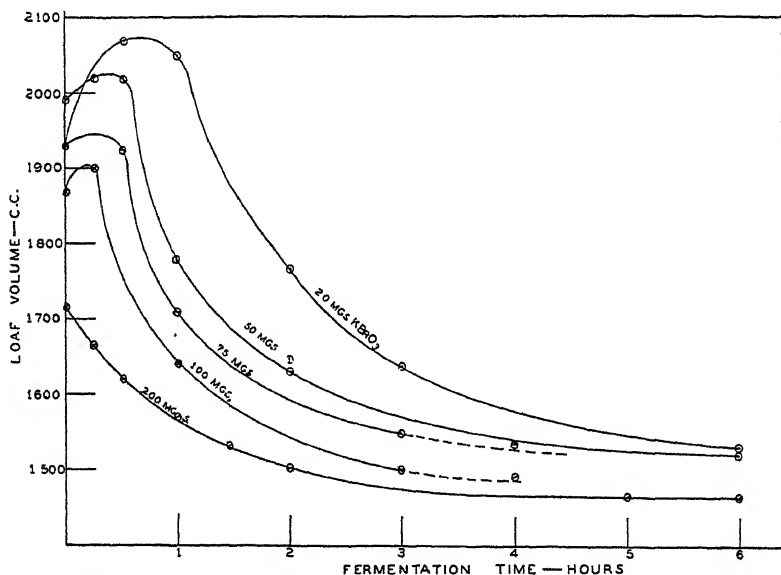


Fig. 5. Effect of fermentation time on loaf volume in doughs with increasing amounts of bromate.

3. There is a tendency for bromate to improve loaf volume; this effect decreases with increasing amounts of bromate and is also affected by the amount of fermentation.

4. The decreases in volume approach a limiting value which seems to be partly dependent on the volume of the dough at the end of the pan proof period, when the dough is placed in the oven; this value is about 1400 cc. in doughs showing the "excess bromate" effect.

5. The figures for the 20-mg. loaves were different for different lots of flour (Tables III and IV), but the curves were parallel, showing that the reaction was of the same type.

It is believed that the "excess bromate" effect is a result of the combined effects of bromate and fermentation.

### Influence of Fermentation Products in the "Excess Bromate" Effect

To determine whether the "excess bromate" effect was partly due to specific effects of the products of yeast fermentation, pyruvic acid and acetaldehyde, which are considered as intermediate products of alcoholic fermentation,<sup>1</sup> were compared to hydrochloric acid in doughs with and without bromate. The results are given in Table V.

TABLE V

EFFECTS OF PYRUVIC ACID, ACETALDEHYDE, AND HYDROCHLORIC ACID ON LOAF VOLUME AND pH IN DOUGHS WITH AND WITHOUT  $\text{KBrO}_3$

	Loaf volume	pH of bread
	cc.	
Control	2120	5.63
Bromate—20 mg.	1900	5.61
HCl, N/1—5 cc.	1830	5.11
Pyruvic acid—0.45 g.	1860	5.19
Acetaldehyde—0.65 g.	1920	5.23
Bromate—20 mg. plus HCl, N/1—5 cc.	1510	5.07
Bromate—20 mg. plus pyruvic acid—0.45 g.	1560	5.20
Bromate—20 mg. plus acetaldehyde—0.65 g.	1650	5.21

From these limited results it does not appear that pyruvic acid and acetaldehyde would be any more effective than HCl at about the same pH.

Having observed that 20 mg. of bromate produced good loaf volume after a very short fermentation period, experiments were conducted to determine whether the addition of acid to very young doughs would produce the "excess bromate" effects resulting from prolonged fermentation. The results of these tests are given in Table VI.

There is a definite decrease in volume with increasing amounts of added acid in these young doughs, but the volumes are much greater than those of the bread at about the same pH shown in Table III, where the dough acidity was produced by fermentation.

Since  $\text{CO}_2$  is one of the products of fermentation, it appeared desirable to note whether it was a contributing factor in the "excess bromate" effect. Experiments with bromate in young doughs with

<sup>1</sup> A. Harden: New light on the chemistry of alcoholic fermentation, J. Inst. Brewing 39: 644-646 (1933).

TABLE VI

EFFECTS OF ADDED ACID ON LOAF VOLUME AND pH IN DOUGHS WITH 20 MG.  $\text{KBrO}_3$   
Dough time, 30 min.; pan proof time, 70 min.

$n/10 \text{ HCl}$	Loaf volume	pH of bread
<i>cc.</i>	<i>cc.</i>	
0	2160	5.69
5	2180	5.60
10	2140	5.54
20	2070	5.41
30	2020	5.24
40	1900	5.11
50	1850	5.00

With the exception of the last three loaves, textures were all satisfactory, and the loaf having a pH of 5.11 was the first to show a distinct "excess bromate" effect. In contrast to this, the loaf having a pH of 5.62 showed a strong "excess bromate" effect, in the tests in which dough acidity was produced by fermentation (Table III).

added acid were therefore made in which the doughs were mixed in an atmosphere of  $\text{CO}_2$  under slight pressure. Table VII shows the results of these tests. (Figure 6 is a photograph of the Hobart-Swanson mixer, as used for mixing doughs in  $\text{CO}_2$  and other gases.)

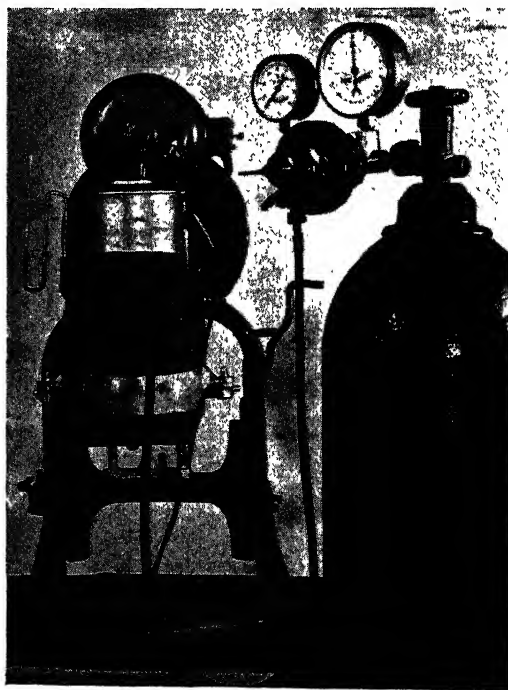


Fig. 6. Hobart-Swanson mixer, as used for mixing doughs in different gases; mixing bowl and mixer head are enclosed in rubber and manometer indicates pressure within mixing chamber.

TABLE VII

EFFECTS OF ADDED ACID AND MIXING IN CO<sub>2</sub> ON LOAF VOLUME AND pH IN DOUGHS WITH 20 MG. OF KBrO<sub>3</sub>

Dough time, 30 min.; pan proof time, 70 to 75 min.

n/10 HCl	Loaf volume	pH of bread
cc.	cc.	
0	2070	5.66
5	2040	5.61
10	1960	5.54
20	1840	5.41
30	1790	5.28
40	1790	5.13
50	1740	5.01

The texture of the first two loaves was satisfactory; the loaf of pH 5.41 was the first to show a distinct "excess bromate" effect.

It is evident that the changes in volume and bread characteristics were very marked, indicating that CO<sub>2</sub> was definitely a factor in the "excess bromate" effect.

Figure 7 shows the changes in volume with decreasing pH resulting

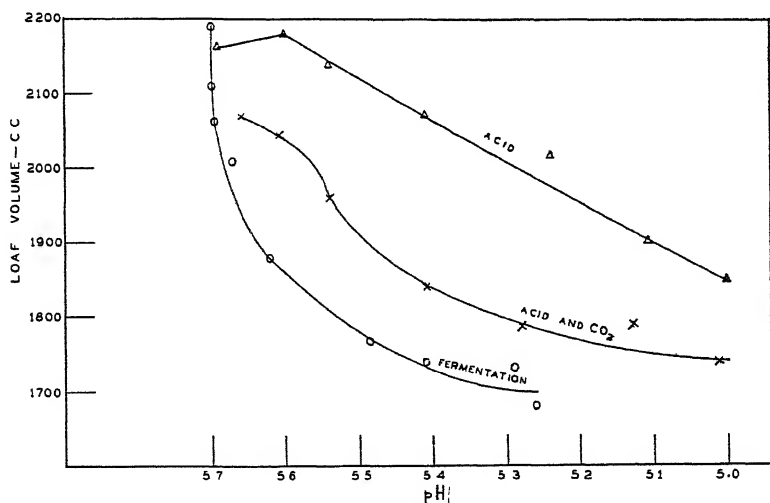


Fig. 7. Comparison between effects of fermentation, added acid, and added acid with mixing in CO<sub>2</sub> on changes in loaf volume due to decreasing pH, in doughs containing 20 mg. of bromate.

from (a) fermentation, (b) added acid, and (c) added acid and mixing in CO<sub>2</sub> (data from Tables III, VI, and VII). It is evident that the curve for acid plus CO<sub>2</sub> is parallel to, but does not coincide with, the fermentation curve, indicating that there may be one or more additional factors involved.

It should be noted that the CO<sub>2</sub>-plus-acid loaves had about the same pH as those with acid alone; CO<sub>2</sub> does, however, produce a difference in pH right after mixing, as shown in Table VIII.

TABLE VIII  
CHANGES IN pH OF FERMENTING DOUGHS MIXED IN AIR AND IN CO<sub>2</sub>

	Dough mixed in air	Dough mixed in CO <sub>2</sub>
pH after mixing	5.98	5.72
pH after 1 hour	5.60	5.65
pH after 2 hours	5.49	5.48
pH after pan proof (3¼ hrs.)	5.29	5.33
pH of baked bread	5.48	5.48

It is apparent that the pH of the dough mixed in CO<sub>2</sub> was much lower than that of the dough mixed in air right after mixing, but this difference disappeared within the first hour, evidently as a result of the production of CO<sub>2</sub> by fermentation in the dough mixed in air.

### Summary

Bromate was found to produce effects in addition to and apparently different from direct inhibition of proteolytic activity.

The acid produced by fermentation in dough was found to be one of the important factors involved in the effects of bromate on the quality of the bread produced.

The carbon dioxide produced by fermentation in dough was found to be another important factor involved in the effects of bromate.

With fermentation time a constant, the effects of bromate were found to vary with the amount of bromate used; this was true even in doughs with very short fermentation periods, indicating that oxidizing agents such as bromate may produce specific effects. To obtain certain reactions of bromate in doughs, fermentation may not be necessary.

# DOUGH OXIDATION AND MIXING STUDIES

## II. EFFECTS OF REMIXING AFTER FERMENTATION

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(Read at the Annual Meeting, May 1938)

It has been stated in Section I of this series that remixing after fermentation produced very marked improvements in doughs which would otherwise produce "excess bromate" effects. Table I shows the changes in volume produced by remixing after fermentation in doughs with (a) different amounts of bromate, (b) different fermentation periods, and (c) very young doughs made with added acid and bromate, which were mixed in CO<sub>2</sub>.

TABLE I  
CHANGES IN LOAF VOLUME PRODUCED BY REMIXING DOUGHS AFTER  
FERMENTATION

Bromate	Loaf volume	Remixed after fermentation— loaf volume
DOUGHS WITH VARYING AMOUNTS OF KBrO <sub>3</sub> ; DOUGH TIME 2 TO 2½ HRS.		
mg.	cc.	cc.
0	1990	1980
5	1900	2160
10	1860	2160
20	1680	2250
40	1640	2210
60	1550	2120
100	1500	2150
250	1480	2170
500	1420	2150
1000	1420	2140
DOUGHS WITH 20 MG. OF KBrO <sub>3</sub> FERMENTED 2 AND 6 HOURS		
	cc.	cc.
Dough time 2 hrs.	1880	2190
Dough time 6 hrs.	1680	2160
DOUGHS WITH 20 MG. BROMATE, 20 CC. $\pi/10$ ACID, MIXED IN CO <sub>2</sub> , DOUGH TIME ½ HR.		
	cc.	
Not remixed	1930	
Remixed after fermentation	2150	

It is evident from these results that remixing after fermentation completely neutralized these "excess bromate" effects, whether produced by large amounts of bromate, prolonged fermentation, or by the addition of acid and CO<sub>2</sub> to a very young dough.

It had been observed that during remixing of doughs with large amounts of bromate, the doughs became very sticky and stringy if



overmixed. Experiments were therefore conducted to determine the effects of variations in remixing time with doughs containing different amounts of bromate. Table II and Figure 1 show the results of experi-

TABLE II

LOAF VOLUME AND pH FOR DOUGHS WITH 0, 20, AND 100 MG. OF  $\text{KBrO}_3$ , REMIXED AFTER FERMENTATION FOR VARYING TIME INTERVALS

Doughs mixed 2 min. and remixed as indicated in Hobart-Swanson mixer—Dough time, 2 hours; pan proof time, 50 to 55 minutes

Remixing time <i>Min.</i>	Loaf volume <i>cc.</i>	pH of bread
DOUGHS WITH NO BROMATE		
$\frac{1}{2}$	1960	5.43
1	2030	—
$1\frac{1}{2}$	2150	5.40
2	2130	—
4	2160	5.38
DOUGHS WITH 20 MG. OF BROMATE		
$\frac{1}{2}$	1720	5.43
1	2050	—
$1\frac{1}{2}$	2190	5.41
2	2230	—
4	2070	5.37
DOUGHS WITH 100 MG. OF BROMATE		
$\frac{1}{2}$	1710	5.43
1	2140	—
$1\frac{1}{2}$	2190	5.41
2	2100	—
4	1770	5.37

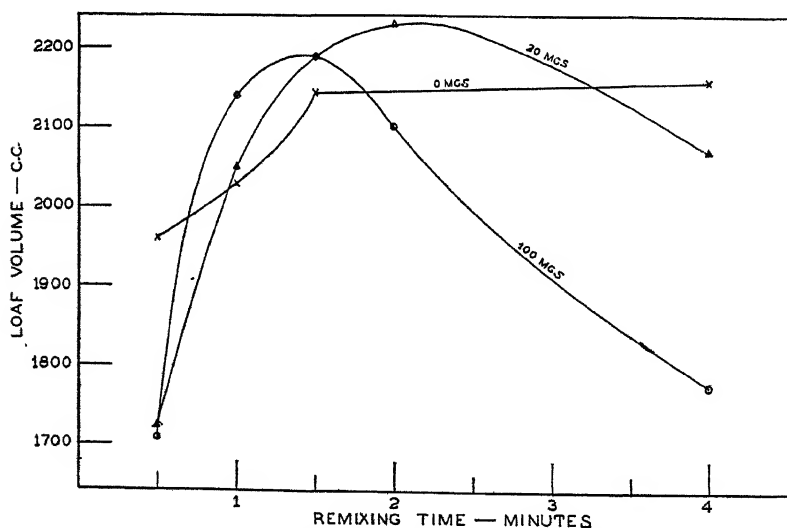


Fig. 1. Effects of variations in remixing time on doughs containing different amounts of  $\text{KBrO}_3$ .

ments with doughs containing 0, 20, and 100 mg. of bromate, and remixed after fermentation for  $\frac{1}{2}$ , 1,  $1\frac{1}{2}$ , 2 and 4 minutes. The doughs were all mixed and remixed in the Hobart-Swanson mixer.

The changes in volume produced by remixing were accompanied by changes in texture and exterior appearance. In the 20-mg. series, the differences were much greater than in the no-bromate series; the  $\frac{1}{2}$ -minute loaf showed a strong "excess bromate" effect; the 1,  $1\frac{1}{2}$ , and 2-minute loaves were about normal in texture though the grain was somewhat open; the 4-minute loaf showed an apparent "proteolytic" effect; that is, the bread was similar in appearance to that made from dough with added papain. We have designated this condition as the "excess-remixing" effect. The loaves in the 100-mg. series were similar to the 20-mg. loaves, but 4 minutes of remixing produced a much stronger excess-remixing effect than in the 20-mg. series.

The differences produced were very striking. A study of the curves led us to the following conclusions:

1. Each curve shows an optimum volume after  $1\frac{1}{2}$  to 2 minutes of remixing time.
2. The optimum for the bromate doughs is greater than for the no-bromate doughs.
3. The initial volume of the bromate doughs is much lower than that of the no-bromate doughs.
4. The change in volume per unit of mixing time increases with the bromate content of the doughs; the 100-mg. curve shows an increase in volume of over 400 cc. due to a change in remixing time of 30 seconds.
5. There was no change in volume after the optimum was reached with the no-bromate doughs, but there was a considerable decrease in volume with the 20-mg. doughs and a very sharp decrease with the 100-mg. doughs.
6. There was a slight but measurable decrease in pH, which varied as the remixing time.
7. The dough with 20 mg. of bromate was sticky and stringy after 4 minutes of remixing, and the 100-mg. dough was very much more so and could not be handled.
8. The loaf made from the 100-mg. dough with 4 minutes of remixing showed a very strong excess-remixing effect.

To determine whether mixing in oxygen initially would produce remixing curves similar to those obtained with bromate, doughs without bromate were mixed in nitrogen, in oxygen, and then remixed in air after fermentation for  $\frac{1}{2}$ , 1,  $1\frac{1}{2}$ , 2, and 4 minutes. The results of these experiments are shown in Table III, with figures for comparable

doughs mixed in air from Table II; in Figure 2 these results are shown graphically.

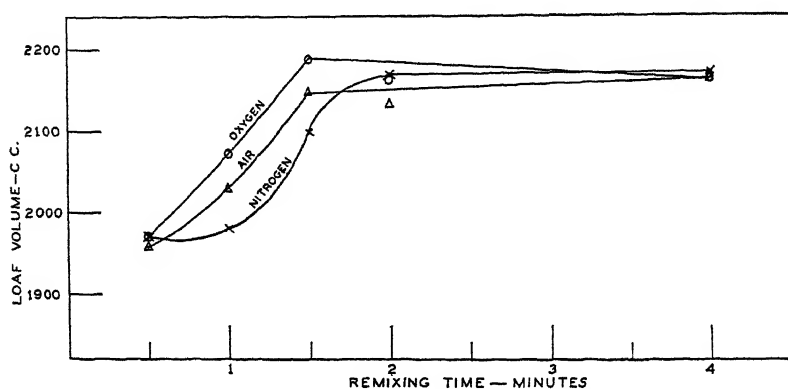


Fig. 2 Effects of variations in remixing time on doughs without bromate, which were originally mixed in nitrogen, air and oxygen.

TABLE III

LOAF VOLUME AND pH FOR DOUGHS WITHOUT BROMATE, MIXED 2 MINUTES IN NITROGEN, AIR AND OXYGEN, AND REMIXED IN AIR AFTER FERMENTATION FOR VARYING TIME INTERVALS

Dough time 2 to 2½ hours; pan proof time, 50 to 55 min.; doughs mixed and remixed in Hobart-Swanson <sup>1</sup>

Remixing time <sup>2</sup>	Loaf volume	pH of bread
<i>Min.</i>	<i>cc.</i>	
DOUGHS WITH NO BROMATE—MIXED IN NITROGEN		
½	1970	5.46
1	1980	—
1½	2100	5.41
2	2170	—
4	2170	5.39
DOUGHS WITH NO BROMATE—MIXED IN AIR		
½	1960	5.43
1	2030	—
1½	2150	5.40
2	2130	—
4	2160	5.38
DOUGHS WITH NO BROMATE—MIXED IN OXYGEN		
½	1970	5.43
1	2070	—
1½	2190	5.42
2	2160	—
4	2160	5.39

<sup>1</sup> Figure 6, Dough Oxidation and Mixing Studies I (page 492), shows the Hobart-Swanson as used for mixing dough in oxygen and nitrogen.

<sup>2</sup> The ½- and 1-minute loaves in the nitrogen series, and ½-minute loaves in the air and oxygen series were not as good as those mixed for longer periods. The latter were about normal in texture, though more open than usual.

The curves in Figure 2 are qualitatively similar to those of Figure 1 only on the side approaching the optimum.

1. Oxygen and air show an optimum at  $1\frac{1}{2}$  minutes of remixing time, but for nitrogen the approach is more gradual, so that the optimum is reached at 2 minutes.

2. The change in volume per unit of mixing time increases with the oxygen content, but is very much less than for the bromate doughs (Fig. 1).

3. There is no decrease in volume after the optimum is reached, in contrast to the bromate doughs, which showed sharp decreases beyond the optimum (Fig. 1).

It is therefore evident that the remixing effects due to the presence of bromate were much more drastic than in the case of those originally mixed in oxygen.

Experiments were now conducted to see what effects oxygen would produce during remixing after fermentation. Doughs with 20 mg. of bromate were mixed in air and then remixed after fermentation in nitrogen and oxygen. The results are given in Table IV, and figures for doughs containing 20 mg. of bromate remixed in air, obtained from Table II, are included for comparative purposes.

TABLE IV

LOAF VOLUME AND pH FOR DOUGHS WITH 20 MG. OF  $\text{KBrO}_3$ , MIXED 2 MINUTES IN AIR, THEN REMIXED AFTER FERMENTATION FOR VARYING TIME INTERVALS IN NITROGEN, AIR, AND OXYGEN

Dough time, 2 hours; pan proof time, 50 to 55 minutes; doughs mixed and remixed in Hobart-Swanson

Remixing time	Loaf volume	pH of bread
<i>Min.</i>	<i>cc.</i>	
DOUGHS WITH 20 MG. BROMATE, REMIXED IN NITROGEN AFTER FERMENTATION		
$\frac{1}{2}$	1750	5.44
1	2040	5.43
$1\frac{1}{2}$	2250	5.43
2	2240	5.40
4	2220	5.40
DOUGHS WITH 20 MG. BROMATE, REMIXED IN AIR AFTER FERMENTATION		
$\frac{1}{2}$	1720	5.43
1	2050	—
$1\frac{1}{2}$	2190	5.41
2	2230	—
4	2070	5.37
DOUGHS WITH 20 MG. OF BROMATE, REMIXED IN OXYGEN AFTER FERMENTATION		
$\frac{1}{2}$	1750	5.40
1	1990	5.39
$1\frac{1}{2}$	2210	5.37
2	2090	5.43
4	1790	5.30

In the nitrogen remix series, the  $\frac{1}{2}$ -minute loaf showed a very strong "excess bromate" effect, the 1-minute loaf was affected less, and the  $1\frac{1}{2}$ , 2, and 4-minute loaves were about normal. In the air remix series, the loaves were similar to the nitrogen loaves, except for the one remixed 4 minutes, which showed an excess-remixing effect. In the oxygen remix series, the  $\frac{1}{2}$ , 1, and  $1\frac{1}{2}$ -minute loaves were similar to the corresponding nitrogen loaves, but the 2-minute loaf showed an excess-remixing effect and the 4-minute loaf a much stronger excess-remixing effect.

The volume figures in Table IV are shown graphically in Figure 3. Figure 4 shows photographs of the loaves remixed in oxygen and nitrogen. These results are just as striking as those obtained with different amounts of bromate.

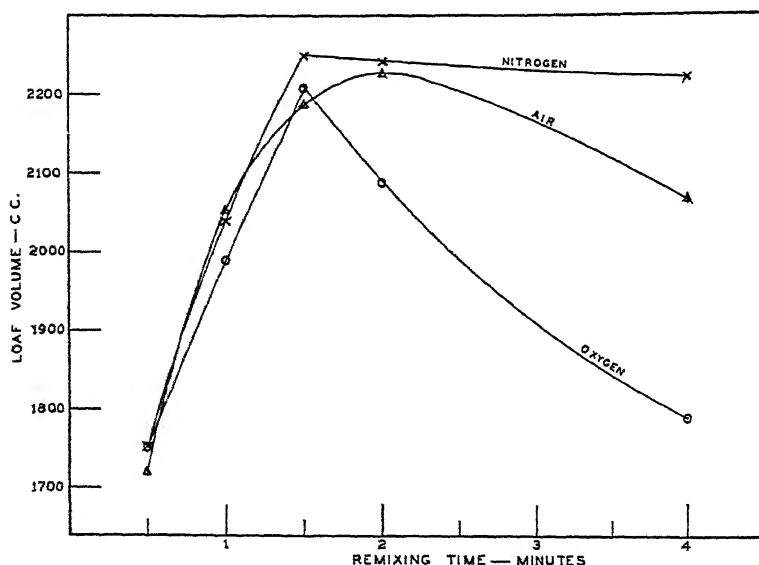


Fig. 3. Effects of variations in remixing time on doughs with 20 mg. of bromate, which were remixed after fermentation in nitrogen, air and oxygen.

1. It is apparent that oxygen plays a decisive role in the volume decreases produced by remixing doughs with bromate beyond the optimum; the absence of oxygen practically eliminates this decrease.

2. It is just as definitely indicated that the influence of oxygen on the sides of the curves approaching the optimum is very slight by comparison.

3. There was a decrease in pH due to remixing in all cases, but the change was definitely greater with oxygen than with nitrogen or air.

4. An excess-remixing effect in the bread, similar to that observed with 100 mg. of bromate and 4 minutes remixing in air, was obtained by remixing 4 minutes in oxygen with 20 mg. bromate. These excess-remixing effects, which resemble the familiar proteolytic effects of papain, are not due to protease action but accompany the physical and chemical changes produced by overmixing. We have been able to show differences in formol titration readings in bread showing proteolytic effects due to papain, but the effects due to overmixing produced no differences in formol titration.

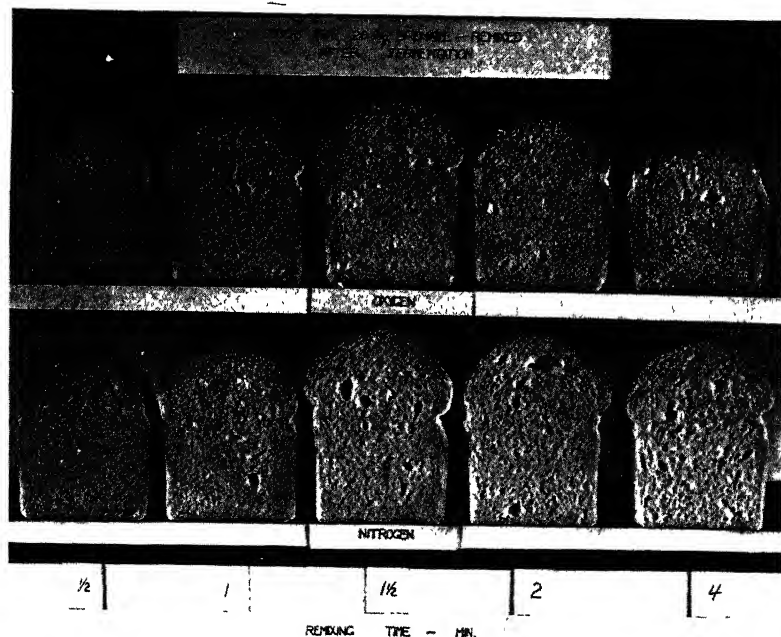


Fig. 4. Photographs of loaves made from doughs with 20 mg. of bromate, showing effects of remixing after fermentation in oxygen and nitrogen, for varying time intervals.

An examination of Figures 1, 2, and 3, side by side, indicates that they all have an optimum loaf volume of roughly 2,200 cc. with a remixing time of  $1\frac{1}{2}$  to 2 minutes. The fact that this common point appears despite the different chemical treatment to which these doughs were subjected, and also the very marked changes in volume produced by as little as 30 seconds difference in remixing time, while approaching the optimum volume, suggest the hypothesis that the volume increases produced by remixing are due mainly to a physical change, perhaps a change in hydration or other colloidal properties of the dough. But

the decrease in volume beyond the optimum may be due to both physical and chemical changes. Evidence of chemical change is indicated by the differences in pH due to incorporated oxygen.

There are at least two practical applications indicated as a result of this work:

1. The production of bread from straight doughs may now be improved, in cases where poor results are obtained as a result of over-fermentation. Given a sufficient amount of sugar in the dough, it may be fermented very much longer than usual and still produce good bread by being remixed for the proper time interval after fermentation.<sup>1</sup>

2. Short-time or "no dough time" bread may be made to resemble bread made in the usual way, by adding acid and oxidizing agent and mixing the dough in carbon dioxide.

### Summary

It was found that the undesirable effects produced by excessive amounts of bromate, or by prolonged fermentation with smaller amounts of bromate, could be eliminated by remixing the doughs after fermentation.

Variations in the remixing time produced great differences in the results obtained; after an optimum remixing time of  $1\frac{1}{2}$  to 2 minutes (in the Hobart-Swanson mixer at 110 r.p.m.), continued remixing produced decreases in loaf volume and bread quality which varied directly with the amount of bromate used in the dough.

The undesirable effects produced by excessive remixing of bromated doughs were found to be due in part to the incorporation of oxygen during remixing and were eliminated by replacing oxygen with another gas such as nitrogen.

It is suggested that the improvements in volume produced by remixing may be due mainly to a physical change in the colloidal properties of the dough.

<sup>1</sup> It has been found that doughs which have been overfermented may be put through the dough break a sufficient number of times, whereby they recover to the point where they will produce good bread. This may be an example of effects similar to those in which the "excess bromate" effect is overcome by the proper remixing interval after fermentation.

## DOUGH OXIDATION AND MIXING STUDIES

### III. THE EFFECTS OF PROTEASES AND REDUCING SUBSTANCES ON DOUGH WHEN MIXED IN OXYGEN

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(Read at the Annual Meeting, May 1938)

In the course of our work with different oxidizing agents, we had occasion to study the effects of oxygen gas on proteolytic activity in dough, and found that by mixing doughs in oxygen, proteolytic activity could be greatly inhibited. A short report on this work has been published (Freilich and Frey, 1937).

#### Effects of Oxygen on Protease Activity

Table I and Figure 1 show the effects on proteolytic activity in dough of mixing increasing percentages of the flour in oxygen.

TABLE I

LOAF VOLUME OF DOUGHS WITH ADDED PAPAIN, AS AFFECTED BY MIXING VARYING PERCENTAGES OF THE FLOUR, WITH WATER, IN OXYGEN<sup>1</sup>

	Loaf volume
	cc.
Papain, not treated	1730
Papain alone treated with oxygen	1780
12½% of the flour, mixed in oxygen	1810
25% of the flour, mixed in oxygen	1860
50% of the flour, mixed in oxygen	1940
75% of the flour, mixed in oxygen	1980
100% of the flour, mixed in oxygen	2100

<sup>1</sup> After the oxygen treatment, the papain and the balance of the dough ingredients were added and the mixing completed in an atmosphere of nitrogen. (Figure 6, Dough Oxidation and Mixing Studies I, shows the Hobart-Swanson, as used for mixing dough in oxygen and nitrogen.)

There was a progressive increase in loaf volume and decrease in the proteolytic effect of the added papain with increasing percentages of oxygenated flour, but the treatment of papain alone with oxygen produced no significant decrease in proteolytic activity.<sup>1</sup>

#### Effects of Oxygen on Reducing Substances

The effects of wheat germ, cysteine, and glutathione are also greatly inhibited by mixing in oxygen, as shown by Table II. Figure 2 shows the effects of oxygen on wheat germ in dough.

The loaves made from the doughs which were mixed in nitrogen

<sup>1</sup> To produce the mixing effects described in this paper a high-speed mixer has been found preferable, though similar results may be obtained by longer mixing in low-speed mixers.



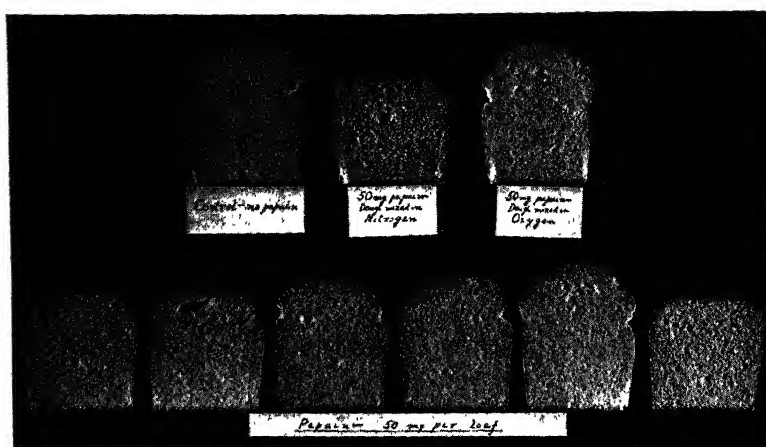


Fig. 1. Effect of oxygen on proteolytic activity in dough. Bottom row shows effects of treating increasing amounts of the flour in water alone (without papain); from left to right the amounts of flour treated with oxygen were 12½%, 25%, 50%, 75%, 100%; the loaf on the extreme right shows that when the papain alone was treated with oxygen outside of the dough, in water solution, its activity was not noticeably affected.

TABLE 'II

LOAF VOLUME OF DOUGHS WITH WHEAT, GERM, CYSTEINE, AND GLUTATHIONE  
Doughs mixed in nitrogen and oxygen

	Loaf volume
	cc.
Control . . . . .	2100
Wheat germ 3%, dough mixed in nitrogen . . . . .	1900
Wheat germ 3%, dough mixed in oxygen . . . . .	2140
Cysteine-hydrochloride 75 mg., dough mixed in nitrogen . . . . .	1850
Cysteine-hydrochloride 75 mg., dough mixed in oxygen . . . . .	2040
Glutathione 50 mg., dough mixed in nitrogen . . . . .	1770
Glutathione 50 mg., dough mixed in oxygen . . . . .	2150

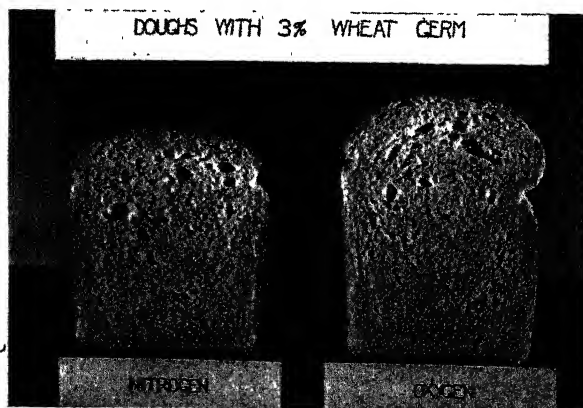


Fig. 2. Effects of mixing in oxygen on dough containing added wheat germ.

showed very strong effects of the proteolytic type, but these effects were almost completely eliminated by mixing the doughs in oxygen. Whether these effects are due to true proteolysis alone remains to be determined.

It should be pointed out that although papain, cysteine, and glutathione apparently produce very similar bread characteristics, their effects in dough are quite different. Cysteine and glutathione produce immediate, specific effects, which are noticeable while the dough is still being mixed. But the effects of papain are very gradual by comparison; doughs with added papain, in amounts which produce effects in bread similar to those of cysteine and glutathione, may be normal after mixing and become soft and sticky only after a few hours of fermentation. Balls and Hale (1936) have made similar observations on the specificity of the effects of cysteine on gluten.

### Effects of Oxygen with Different Types and Grades of Flour

The improvements noted above were obtained with doughs to which wheat germ, cysteine and glutathione and excessive amounts of protease, were added, in order to magnify the differences produced by mixing in oxygen.

TABLE III

LOAF VOLUME OF BREAD MADE FROM DIFFERENT TYPES OF FLOUR—DOUGHS MIXED IN NITROGEN, AIR AND OXYGEN  
Mixing time, 2 min. in Hobart-Swanson

	Loaf volume
	cc.
NORTHWESTERN SPRING STRAIGHT FLOUR	
Dough mixed in nitrogen	1970
Dough mixed in air	1980
Dough mixed in oxygen	2120
Dough mixed in oxygen <sup>1</sup>	2120
NORTHWESTERN BAKER'S FLOUR, TYPICAL SPRING WHEAT	
Dough mixed in nitrogen	2010
Dough mixed in air	2050
Dough mixed in oxygen	2080
Dough mixed in oxygen <sup>1</sup>	2100
SHORT PATENT, SPRING WHEAT FLOUR	
Dough mixed in nitrogen	2050
Dough mixed in air	2050
Dough mixed in oxygen	2030
Dough mixed in oxygen <sup>1</sup>	2040

<sup>1</sup> Dough was kept in mixer in an atmosphere of oxygen for a total of 5 minutes, but the mixing was broken up into four 30-second intervals, with three 1-minute rest periods in between, in order to prolong the oxygen treatment without changing the mixing time of 2 minutes.

Experiments were also conducted with different types and grades of flour, in order to see how far their baking quality could be improved by mixing in oxygen and to compare such improvements with those obtained when making deliberate changes in baking quality by additions of papain or wheat germ. In Table III and Figure 3 are shown loaf volumes and photographs of bread made from different types of flour and mixed in nitrogen, air, and oxygen.

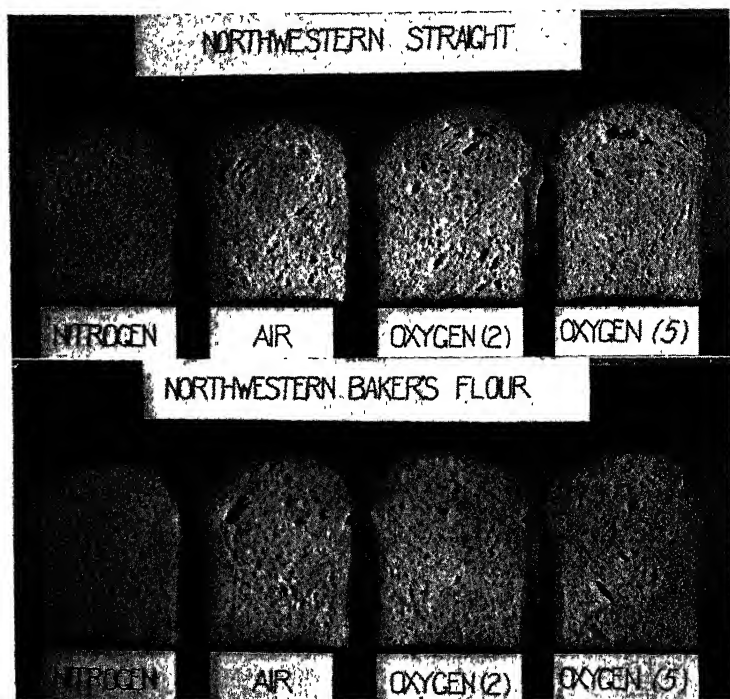


Fig. 3. Flour mixed with increasing amounts of oxygen; last loaf treated with oxygen 5 min., as explained at bottom of Table III.

Mixing with increasing amounts of oxygen produced progressive and decided improvements in the texture and also the crumb color of bread from the Spring Straight and the Northwestern Baker's flour; distinct improvement in volume was obtained with both flours but was greater with the Spring Straight. There were no improvements in volume or texture with the short patent, but a distinct improvement in crumb color was apparent.

In Table IV and Figure 4 are shown loaf volumes and photographs of bread made from the different fractions of flour milled from a good grade of Texas wheat.

TABLE IV

LOAF VOLUME OF BREAD MADE FROM THE DIFFERENT FRACTIONS OF FLOUR MILLED FROM THE SAME WHEAT—A GOOD GRADE, HIGH PROTEIN, TEXAS WHEAT  
Doughs mixed in nitrogen, air, and oxygen; mixing time 2 min., in Hobart-Swanson

	Loaf volume
	cc.
100% EXTRACTION FLOUR	
Dough mixed in nitrogen	2000
Dough mixed in air	2000
Dough mixed in oxygen	2070
Dough mixed in oxygen <sup>1</sup>	2100
PATENT FLOUR	
Dough mixed in nitrogen	1870
Dough mixed in air	1980
Dough mixed in oxygen	2010
Dough mixed in oxygen <sup>1</sup>	1980
FIRST CLEAR FLOUR	
Dough mixed in nitrogen	1700
Dough mixed in air	1820
Dough mixed in oxygen	1920
Dough mixed in oxygen <sup>1</sup>	1910
SECOND CLEAR FLOUR	
Dough mixed in nitrogen	1690
Dough mixed in air	1810
Dough mixed in oxygen	1930
Dough mixed in oxygen <sup>1</sup>	1980

<sup>1</sup> Dough was kept in mixer in an atmosphere of oxygen for a total of 5 minutes, but the mixing was broken up into four 30-second intervals, with three 1-minute rest periods in between, in order to prolong the oxygen treatment without changing the mixing time of 2 minutes.

Mixing the doughs with increasing amounts of oxygen produced distinct improvements in volume and great improvements in texture and crumb color with the 100% extraction and the patent flours. But the improvements were even greater with the first and second clear flours; the loaves produced by mixing in nitrogen and air from these flours were poor in volume, color, and texture, and decidedly unsatisfactory, but the loaves produced by mixing in oxygen were very much better in all respects, and the textures were practically normal.

It is evident that the lower the grade of the flour, the greater is the improvement produced by mixing in oxygen. The improvements with the first and second clear flours are comparable in magnitude to the differences obtained when adding papain or wheat germ to the dough and mixing in oxygen.

In Figure 5 are shown loaves made from the second clear flour used above; a commercial type formula, including malt extract and powdered milk, was used; the small loaf was mixed in nitrogen, and its volume was 1,910 cc.; the large loaf, with a volume of 2,130 cc., was mixed in oxygen; the great improvement produced by oxygen is quite apparent.

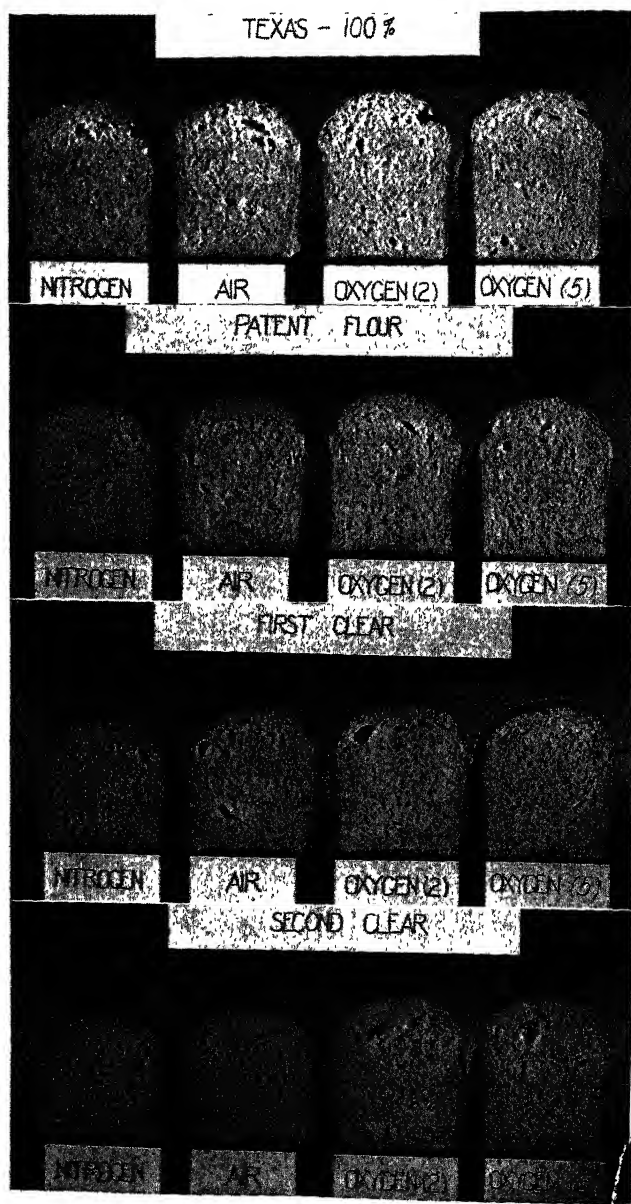


Fig. 4. Loaves made from the same Texas wheat. Flours mixed with increasing amounts of oxygen.

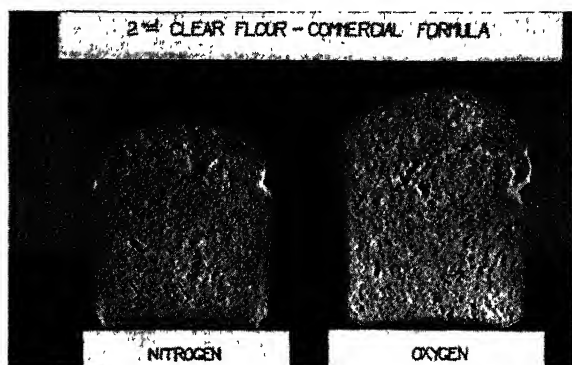


Fig. 5. Effects of oxygen on 2nd clear flour in a commercial formula.

### Formol Titration Experiments

We have seen that the activity of added papain is greatly retarded by mixing doughs in oxygen and also that definite improvements in bread quality are obtained by mixing doughs from different flours in oxygen. To determine whether these improvements may be due, at least in part, to the inhibition of the activity of native protease present in flour, we made formol titrations on extracts of bread made from doughs which were allowed to stand for different time intervals, after mixing in oxygen and nitrogen, with and without added papain. Patent flour was used. We assumed that increases in the amount of formol nitrogen were due to proteolytic activity. The results are shown in Table V.

TABLE V

FORMOL TITRATION RESULTS, WITH BREAD EXTRACTS FROM DOUGHS WITH AND WITHOUT PAPAIN, MIXED IN NITROGEN AND OXYGEN<sup>1</sup>

	Formol $N^2$ (cc. $n/200$ NaOH per 25 cc. bread extract)		Increase in formol $N$ (cc. $n/200$ NaOH)
	$\frac{1}{4}$ -hr. dough	18-hr. dough	
Control, mixed in oxygen	3.1	4.2	1.1
Control, mixed in nitrogen	3.4	5.95	2.55
Papain, 50 mg. mixed in oxygen	4.9	7.6	2.7
Papain, 50 mg. mixed in nitrogen	5.45	17.1	11.65

<sup>1</sup> Ninety percent of the flour and all other ingredients except the yeast were made into doughs and allowed to stand for  $\frac{1}{4}$  hr. or 18 hrs., then they were remixed with 3% yeast, the balance of flour and water, allowed to stand for a few minutes, then rounded, proofed and baked.

<sup>2</sup> Method for formol titration: To 50 g. bread crumb, added 150 cc. water, stirred, centrifuged, and used 25 cc. portions of the liquid for formol titration as in the usual procedure, but with the following modifications: used  $n/200$  NaOH, and electrometric titration with glass electrode to obtain better end point than with phenolphthalein; titration error,  $\pm 0.1$  cc.

These results show a great increase in formol N due to papain and a definite increase in the doughs without papain. These increases were greatly reduced by mixing in oxygen. This indicates that (a) doughs made with patent flour show evidence of proteolytic activity, and (b) this activity is greatly retarded by mixing the doughs in oxygen.

### Discussion

There has been some discussion regarding the explanation of the improvements produced in bread making by the use of oxidizing agents such as potassium bromate (Jørgensen 1935; Read and Haas 1937). In view of the inhibition by bromate and by oxygen of the effects of papain and the inhibition by oxygen of the production of formol N in dough, it is not illogical to ascribe the improvements produced by oxidation, at least in part, to the inhibition of protease activity in dough.

But if the effect of bromate were merely to inhibit protease activity and nothing more, then there should be no further effects, after an amount of bromate sufficient to accomplish this purpose has been added. However, it is evident from some of our work that the effects of bromate and other oxidants are not confined simply to inhibition of proteases—both added and those present in the flour—but are far more profound, as indicated in Section I of this series. These latter effects must also be taken into account in seeking an explanation of the effects of oxidation in dough. It remains to be established to what extent these and perhaps other factors are effective under ordinary conditions of bread making.

The inhibition of proteolytic activity by oxygen incorporated into dough during mixing is an effect very much like that of bromate in the presence of added papain; our work with oxygen seems to indicate action on some flour constituent rather than direct action on the enzyme by oxygen.

The improvements produced by oxygen with different flours, particularly lower-grade flours, may be due not only to inhibition of protease activity, but also to oxidation of reducing substances, such as glutathione. Our results and those of Balls and Hale (1936) show that cysteine and glutathione produce very harmful effects in bread, and Sullivan, Howe, and Schmalz (1936) have shown the presence of glutathione in wheat germ. We have shown that the harmful effects produced by additions of cysteine, glutathione, and wheat germ were partly overcome by mixing the doughs in oxygen. The lower the grade of flour, the greater is the concentration of protease and germ fractions in it; consequently the improvement produced by oxygen is

greater. But the improvement produced by oxygen even with normal bread flours is by no means negligible, and may be explained on the same basis.

It has been shown that oxygen produced both detrimental and good effects in bread making, and it may be well to point out that there is no contradiction involved, since the conditions under which these effects were obtained were entirely different. The detrimental effects<sup>2</sup> were produced by too much remixing, after fermentation, in doughs containing bromate; the good effects were obtained in doughs without bromate, which were originally mixed in oxygen and not remixed after fermentation.

The following brief description may give a more coherent picture of the relationships involved in the results of the work presented in these papers. The addition of protease or glutathione to a dough, which would ordinarily give a normal loaf of bread, produces bread of low volume and poor quality. The effects of protease or glutathione may be overcome and normal bread again obtained by adding the proper amount of oxidizing agent such as  $\text{KBrO}_3$ , or by mixing the dough in oxygen. The addition of too much bromate, however, produces a poor loaf, showing the "excess bromate" effect. This in turn is overcome and normal bread obtained by remixing the dough for the proper time interval after fermentation. But too much remixing in air or oxygen breaks down the dough and gives poor bread. By replacing the air or oxygen with another gas such as nitrogen, the effects of excessive remixing may also be overcome, and normal bread again obtained. This is shown diagrammatically, in Figure 6.

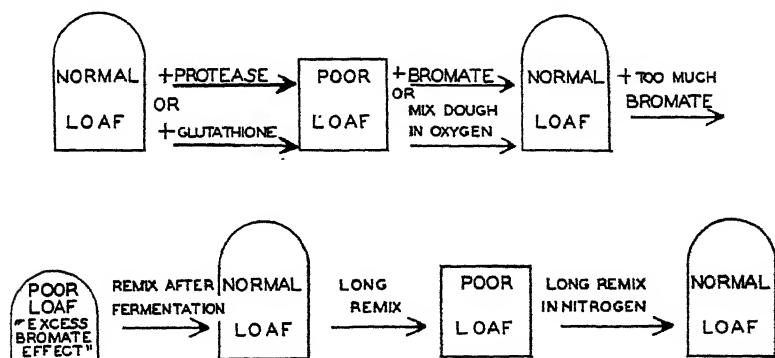


Fig. 6. Diagrammatic representation of interrelationships of oxidation and mixing in bread doughs.

<sup>2</sup> Dough Oxidation and Mixing Studies II.



The importance of these observations on the effects of oxygen may not be confined entirely to the baking field. It may find application in studies on the germination of seeds, reactions in normal and abnormal animal tissue such as cancer cells, proteolytic action of bacteria and molds, and the autolysis of tissues.

### Summary

The effects of papain in dough were greatly retarded by mixing the dough in oxygen.

The effects of oxygen were found to be due to its action on the flour, and not to any direct action on the papain.

The effects of wheat germ, cysteine, and glutathione were greatly retarded by mixing in oxygen.

Great improvements in volume, color, and texture were produced by mixing doughs from different types of flour in oxygen; the greatest improvements were obtained with the lower grades, such as first and second clear flours.

The production of formol N in doughs made from regular white bread flour was markedly retarded by mixing the doughs in oxygen.

### Acknowledgment

We wish to acknowledge the assistance and advice of Dr. Quick Landis.

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# A METHOD AND APPARATUS FOR TESTING DOUGHS

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(Read at the Annual Meeting, May 1938)

During one of the investigations in this laboratory an attempt was made to produce crustless bread. It appeared probable that internal heating of the dough should produce such bread. Heat can be generated internally by using the dough as the resistance between electrodes which are carrying alternating current. By this method crustless bread was produced which had a thin skin on all exposed areas. The final bread appears nearly as though the crust had been cut away from a sandwich loaf. This method of making bread made it appear possible to test the properties of the dough during proofing and baking. Loaf volume can be measured during oven spring by observing the rise of a pointer supported on a light disc resting on the top of the dough. The resistance to the movement of a plunger through dough can be measured by having a pointer attached to a weight and following its progress as it sinks into the dough during baking. The downward movement of a plunger in a loaf during baking is complicated by the upward movement of the dough due to oven spring. This carries the plunger in an opposite direction, thus requiring a rather elaborate method of correction so as to arrive at a true picture of the plunger's passage through the dough. The indications of the plunger cease during dough thickening so that no further information regarding the character of dough is obtained.

In order to cook a dough and have a controlled rate of temperature rise in the dough while these tests are being carried on, it is desirable that the heat input into the dough be constant at all times throughout the cooking. This result can be accomplished by applying a constant wattage to the dough, using a watt meter and a variometer for control. Also a volt meter can be placed in the line and the significance of variation in electrical resistance of the dough can be gained by reading the changes in voltage that are required to keep a constant wattage flowing through the dough, thus giving an additional test indicating the conductivity of the dough while baking.

If one could measure the pressure in the cellular structure of dough while proofing and baking, that measurement should indicate the stress which the dough is under at any particular moment. It was found that the pressure within the dough can be measured during these bakings by forming a small chamber of relatively large area underneath the dough, which is sealed around the edges to avoid leaks, and which

communicates to a manometer of fine bore. Under this condition the gases in the dough diffuse into this chamber and build up a pressure which is in equilibrium with the pressure within the dough. To produce this pressure the transfer of only a small amount of gas is required. The sensitivity of the method is evidenced by the pressure responding at once to changes in the rate of heating. Also the reading drops to zero immediately upon cutting off the heat, thus showing that the pressure within the chamber is in equilibrium with the pressure within the dough. The chamber is constructed of a fine-mesh metal screen which is insulated by a proper coating and is further covered with a thin layer of glass wool. The glass wool gives better insulation and more reproducible pressure changes.

The complete device is shown by Chart I in two sectional views.

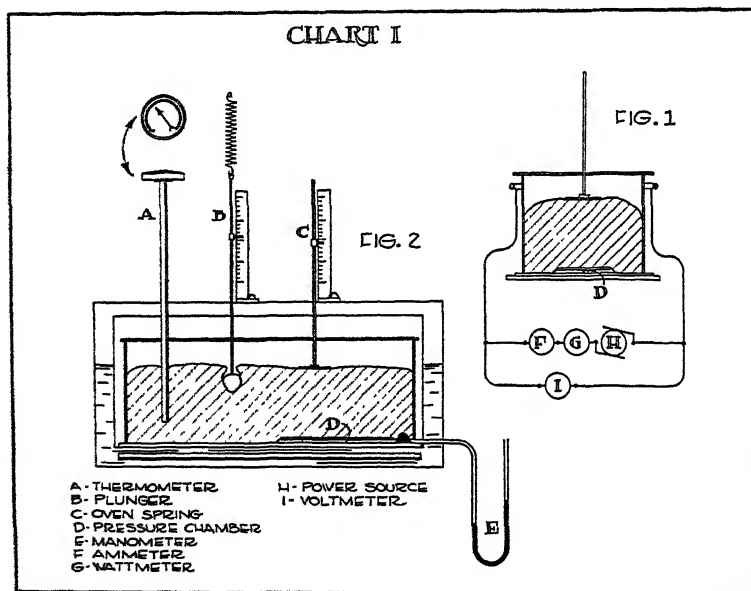


Figure 1 gives an end view cross-section of the baking pan showing the insulated bottom and the electrodes on the sides with electrical connections for carrying the current. The electrical instruments in the circuit and the float for loaf volume are shown. Underneath the dough is the flat pressure chamber formed by a screen and glass wool with a small-bore pipe leading out to a manometer, which is shown in the side view in Figure 2. In order to avoid condensation of moisture on the inner side of the walls of the pan as heat is generated in the

dough, it is desirable to either insulate<sup>1</sup> or keep the pan at a slightly elevated temperature above that within the dough. This latter is accomplished by placing the pan in a chamber which is heated. The chamber can be heated to approximately the boiling point of water without introducing any serious error into the readings, but it is more desirable that the chamber be heated progressively as the dough is heated but more rapidly, so that a small transfer of heat to the pan occurs to prevent condensation on the walls.

The various measuring instruments are shown in their position. Readings are taken regularly, as nearly simultaneously as possible, so that the records will show all properties of the dough at the same instant.

It is desirable to heat at approximately the same rate as a loaf of bread is heated in the oven. Of course, one must recognize that the result of heating dough interiorly is different from that which takes place in the commercial baking of bread, where the heat rise progresses from the outside to the inside. However, each portion of the dough in crust bread must go through a similar cycle of heating as does the dough in the electric pan. Thus the reactions which are observed in the electric pan can be construed to have occurred in any one portion of a commercial loaf, the main difference between the two baking methods being the rate at which the reactions happen respectively in the different zones and the effect of these reactions upon each other.

It is to be noted that the plunger has a spring attached to it, so that as it sinks through the dough its effective weight is reduced. The use of the spring was found desirable to enable one plunger to operate properly in all characters of dough. A weight which would sink slowly through a soft-wheat dough would not penetrate a strong hard-wheat dough, and *vice versa* a weight which would penetrate a strong dough would sink immediately to the bottom in a soft dough. By supporting the weight with a spring so that its effective weight becomes less as it sinks, its application can be made universal and the results made comparable. However, it has been found in practice that the use of the plunger in conjunction with the use of the pressure chamber on the same dough presents some undesirable features because the penetration of the plunger into the dough during the oven spring affects the pressure within the dough and interferes with correct readings in the pressure chamber; hence in many instances it is desirable that these readings be taken separately on duplicate doughs of the same kind. It has also been found difficult to duplicate plunger readings. Further changes in this feature are being made. The results of all the readings

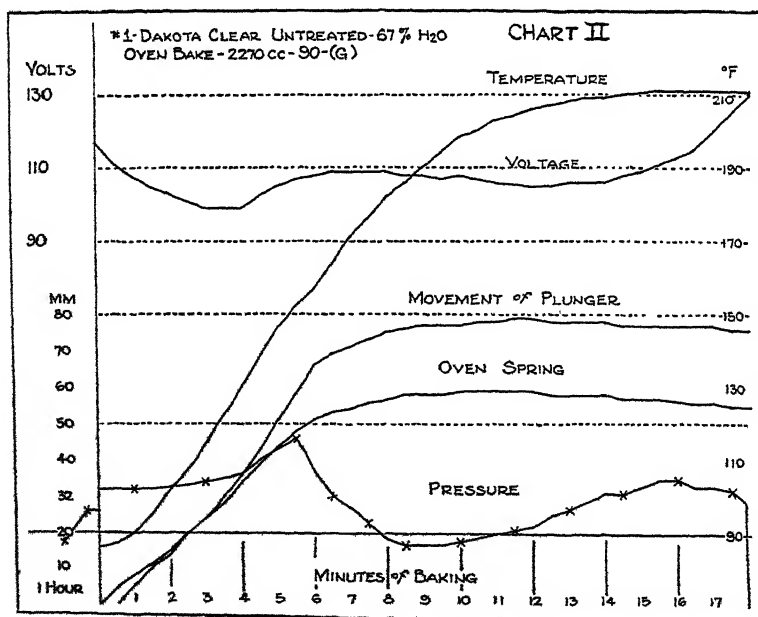
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<sup>1</sup> The construction and technique of using an insulated pan will be further described in a later publication.

are plotted on one chart with time as the base (or abscissa) and the movement or pressure in millimeters as the ordinate. In the electric measurements, the voltage of the current is the ordinate. On the chart one can then see the record of any or all of these properties at the same instant, as well as follow their progress during the course of baking.

To start the operations the dough is handled as usual and proofed in the electric pan in the proof box in the regular way. The manometer is attached to the pressure chamber while proofing so that the pressure of proofing can be read. When standard proof height is reached the pan is transferred, with manometer still attached, to the testing chamber where the thermometer is inserted in the dough and the loaf volume float is set in position on the dough and the plunger lowered to the dough surface. The current is then turned on and the plunger released simultaneously and all readings taken immediately and continued at regular intervals until 212° F. or the highest voltage available is reached, when the test is discontinued.

Chart II gives the results obtained on a freshly milled flour using 540 grams of dough in a  $4\frac{1}{2} \times 9$ -inch pan, and shows how the various measurements are recorded so their relation to each other can be seen at any moment during the baking process.



### Summary

A method has been shown for baking doughs by making them the resistant unit between electrodes carrying alternating current.

Means are also shown for determining the voltage required to keep a constant wattage flowing through the dough, the rate of temperature rise produced by the heat generated from the current, and the rate the dough rises as the heat is generated. Special means are shown for determining the rate at which a heavy plunger will pass downward through the dough while oven spring is occurring. Also, special means are shown for determining the pressure within the dough throughout the entire baking period.

By preventing evaporation, the formation of a crust is avoided and the properties determined are those produced by the dough itself independent of the influence of a crust.

## EFFECT OF TEMPERATURE ON DOUGH PROPERTIES, I

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(Read at the Annual Meeting, May 1938)

A method and apparatus for testing the properties of bread dough while being baked by internal heating, as described by Baker in the previous paper, has been tested by us to obtain some idea of its significance and application. The method indicates the temperature rise of dough during baking, the voltages applied to maintain a predetermined wattage within the dough, the fall of a plunger through the dough while baking, the gas pressures generated within the dough, and the oven spring. These values can be obtained simultaneously, so that their relation can be readily noted. They will be discussed separately and then in combination.

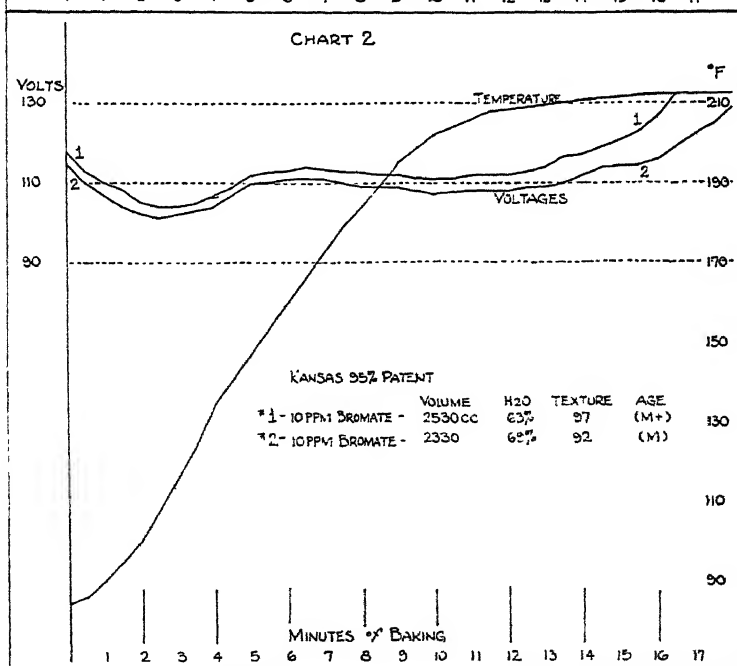
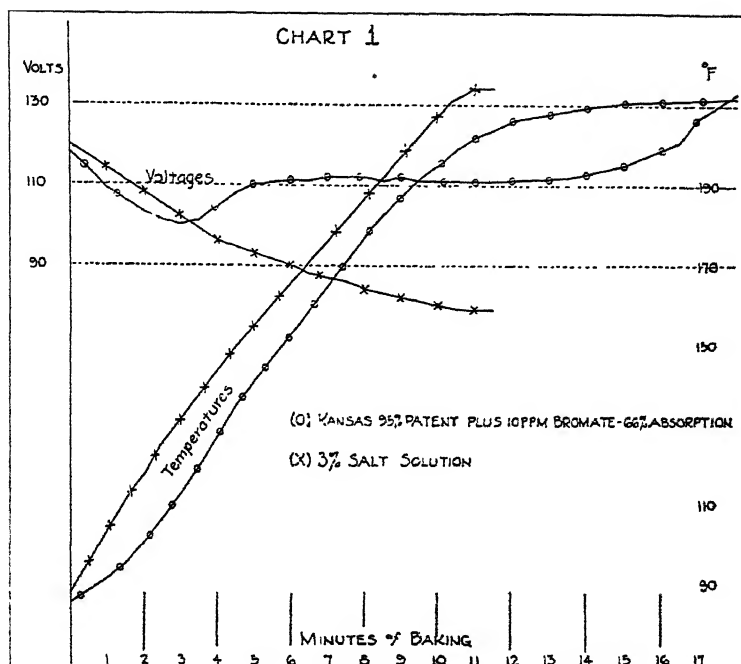
Chart 1 shows the rise of temperature at the center of the loaf while being baked with heat generated from 200 watts passing through 540 grams of dough in a pan  $4\frac{1}{2} \times 9$  inches. The uniform generation of heat within a substance which undergoes no chemical or physical change should cause a regular increase in temperature if no heat were added or subtracted. Such a temperature curve when plotted against time would be substantially a straight line. Any deviation from a straight line in such heating of dough indicates absorption of heat by some physical or chemical change in the dough mass. It will be seen that fermented dough, while heating at a uniform heat input, does not warm with a uniform temperature gradient (Bailey and Munz, 1938),

there being periods where there is a slower temperature rise than at other times, indicating an increased absorption of heat during these periods. There is a noticeable absorption of energy at the beginning of the heating period, which continues up to about 118° to 120° F. This heat absorption is believed to be due to carbon dioxide coming out of solution in the dough and from any loosely bound chemical combination it may have with the dough ingredients. The temperature then climbs more rapidly until approximately 130° F. is reached, after which the rise is slightly less. This change coincides with starch swelling and may be due to that reaction. The rate then continues uniformly until about 175° F. is reached, where there is further absorption of heat which becomes progressively greater until the end of the heating at 212° F. The large heat absorption toward the end of the cooking period is believed to be due primarily to the evaporation of alcohol and water. It is apparent that alcohol acts as a buffer to temperature rise in a dough, interfering with its approach to the boiling point of water. This helps explain why commercially baked bread does not reach 212° F. in the interior of the loaf (Bailey and Munz, 1938).

The effect of alcohol evaporation on the temperature curve is greater than that of carbon dioxide, as one would expect, because of its larger amount (Blish and Hughes, 1932), liquid state, and greater solubility in the dough. The temperature curve offers the interesting suggestion that there are two sources of pressure in a loaf during baking. Alcohol vapor is available in greater potential volume than carbon dioxide and exerts its pressure after the temperature of the dough exceeds the boiling point of alcohol. The significance of this will be considered later in connection with pressure studies.

Chart 1 also shows the voltages which were required to maintain a current of 200 watts in the dough during the entire period of cooking. During the heating of bread dough the voltage drops while the dough warms to approximately 120° F.,<sup>1</sup> when its downward course ceases and the voltage rises rather sharply as the dough warms until between 145° and 165° F. is reached. The swelling temperatures of wheat starch (Dedek, Jelinek, and Kulcickyj, 1936; Cook and Axtmayer, 1937) and the setting range of dough occur during this voltage rise, suggesting that here water is being taken up by the starch. The voltage now remains substantially at a constant level for a sustained period, when a further marked rise occurs to the end of the experiment. It is not possible to continue the current after 212° F. is reached, as

<sup>1</sup> It has subsequently been found that under the conditions of these experiments the temperature of the dough was not uniform, being warmer near the electrodes due to local resistance at the electrode surface. Also a thermometer follows the temperature of a rapidly heating body with an appreciable lag. Subsequent work in which these errors are largely eliminated shows that this phenomenon occurs at 130° F. or higher.





evaporation of water occurs at the electrode surface and rapidly builds up resistance in the circuit. A maximum of 130 volts was usually sufficient to raise the dough to approximately 212° F.

The voltage changes and temperature rise of a 3% salt solution heated in the same manner as is the dough are also shown. This is not a straight line, as adiabatic conditions were not maintained during the heating.

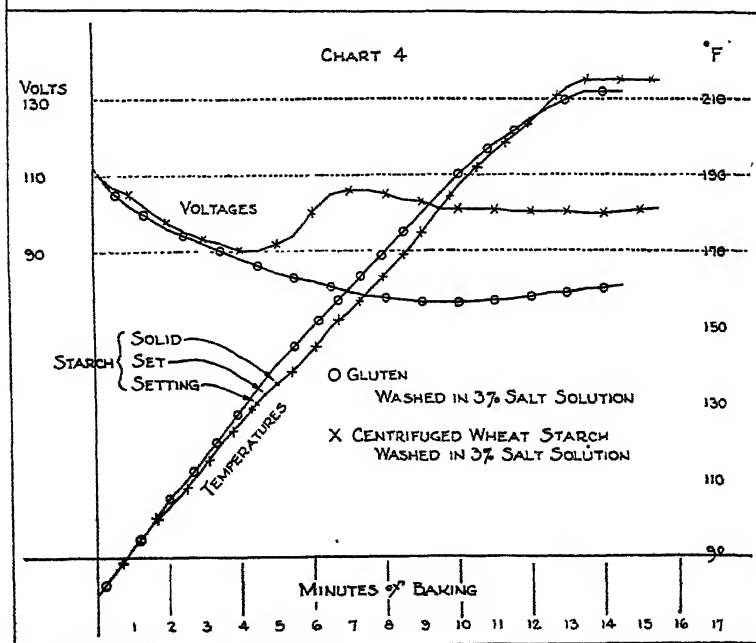
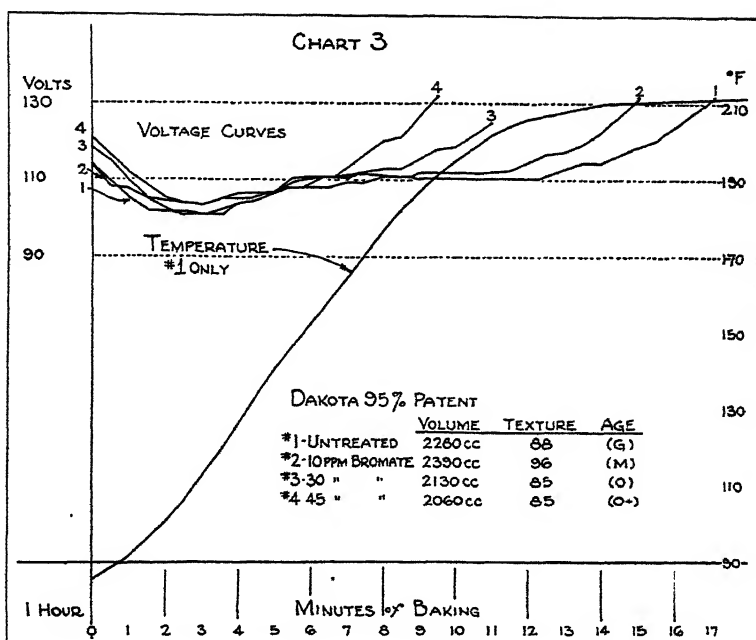
Chart 2 shows the effect upon the voltage of changing the absorption water in the dough by 6%. Of the two voltage curves given, the one with the lower voltage is for the higher absorption, namely 69%, and the one with the higher voltage is for the 63% absorption, indicating that the voltage level is controlled by the dough's conductivity; thus less water, higher resistance.

Chart 3 shows the voltage curves where the doughs being baked received different degrees of oxidation with potassium bromate. The higher treatments cause the final voltages to rise sooner than the lower treatments, suggesting that the bromate treatment accelerates some reaction in the dough that increases its electrical resistance.

A most striking thing about these curves is the shift in the final voltage rise; this rise occurs at a lower temperature for every increment of bromate, until the highest treatment of bromate has caused the start of the rise to change from 210° F. to approximately 160° F. Apparently this increase in resistance of the dough occurs independently of alcohol evaporation. In view of the fact that the starch has largely swollen prior to this rise, it would appear likely that some reaction of the gluten or some electrode phenomenon might also account for such a large increase in voltage. Further investigations on this point are under way.

Chart 4 shows the voltage and temperature curves for starch and gluten separately heated in 3% salt solution in the same manner as described for dough. The starch was prepared by washing it from the dough with a 3% brine, centrifuging the starch from the brine, and packing the moist starch into a cell between electrodes. This gives a concentration of starch slightly higher than is obtained in commercial doughs. The gluten from this washing was collected, and after removal of all the excess brine, was packed in a cell in a similar manner. This gluten was necessarily more concentrated than in doughs. The voltage and temperature changes were then noted while the current was applied.

While the starch was heating a drop in voltage occurred, followed by a rise during the period of thickening, similar to the behavior of dough. After the thickening of the starch had solidified the mass, the voltage for the starch resumed its downward course as does the



salt solution. The temperature rise of the starch is fairly regular throughout the entire course of the heating to 212° F. The voltage required to heat gluten behaves at first in a similar manner to the salt solution but later drops less rapidly, indicating some reaction in which heat is absorbed.

Chart 5 shows the progress of the plunger through doughs while cooking. The weight at first sinks at a moderate rate. As the dough springs upward, however, the weight penetrates more easily and falls very rapidly just prior to the period of starch swelling. Upon the dough becoming thick around 150° F. the plunger's further motion is almost completely arrested. The period of very rapid motion may be due in part to a softening of the dough (Bailey and Munz, 1938) but is probably also influenced by the decrease in density of the dough due to oven spring and by the approach of the dough cell walls to the limit of their tensile strength, making them more readily broken by the additional strain furnished by the moving weight. This is always the character of the plunger's motion except in unoxidized doughs from freshly milled flours or weak flours. In these cases the weight will plunge to the bottom of the dough very rapidly with little alteration of its speed.

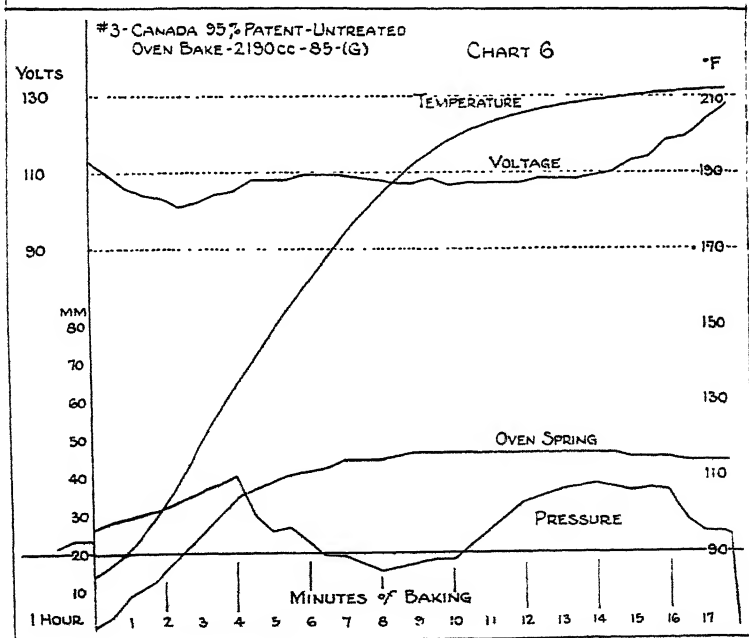
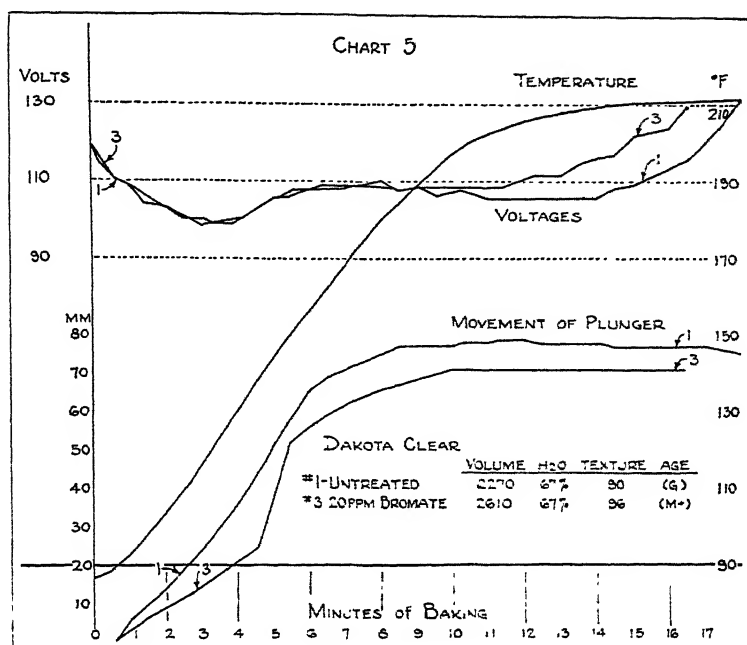
Plunger measurements are not included in the remaining charts as their duplication has been found somewhat difficult and our technique is not considered to have reached a stage where we can rely upon this measurement. Further improvement of this technique is under way.

Chart 6 shows the results obtained from measuring the pressure in the chamber under the loaf during proofing and baking. The weight of dough above the pressure chamber, with 540 g. of dough spread over a pan  $4\frac{1}{2} \times 9$  inches is such that it will require a pressure of approximately 20 mm. of oil of 0.85 density to overcome the weight of the dough. This will be referred to in this paper as the "hydrostatic pressure" and is indicated by a black line across the bottom of the chart at the 20-mm. position.

The charts also have a vertical line separating the first major division from the latter portion of the chart. This first division represents one hour of time and is used to record the pressures obtained during proofing. The remainder of the chart is divided into 2-minute divisions for each corresponding section, so that the entire chart from this point covers a period of 18 minutes,<sup>2</sup> during which the baking of the loaf is carried to 212° F.

While proofing, the pressure in the dough rises until nearly a constant level is obtained during the last 15 minutes. This is usually somewhere between 22 and 30 mm. of oil, and indicates the force

<sup>2</sup> The time of heating can be varied to suit the experimenter by altering the wattage.



required within the dough at the bottom of the pan to expand the dough at the rate of proofing. By subtracting the hydrostatic pressure from these values, an indication of the stress on the bubbles producing a strain in the dough can be obtained. When the dough is heated in the oven an abrupt rise in pressure immediately occurs, indicating the stress required to expand the dough at the approximately ten times faster rate of rise obtained when baking.

Also shown are the voltage, temperature rise, pressure variations, and oven spring of this unoxidized dough while being heated in the electric pan. The pressure slowly rises during the first few minutes of heating, then more rapidly to a peak, which often coincides with the zone of starch swelling, followed by an abrupt and large fall in pressure to below the hydrostatic level of the pressure of the dough, followed by a second rise during the alcohol distillation period to a second peak of approximately the same height as the first, and finally falling off as the heating approaches 212° F. with an abrupt fall to zero when the current is cut off. It is interesting to note that this abrupt fall upon cutting off the current indicates that the pressure in the chamber is in communication with the interior of the loaf and is evidence that these measurements indicate the pressure within the loaf.

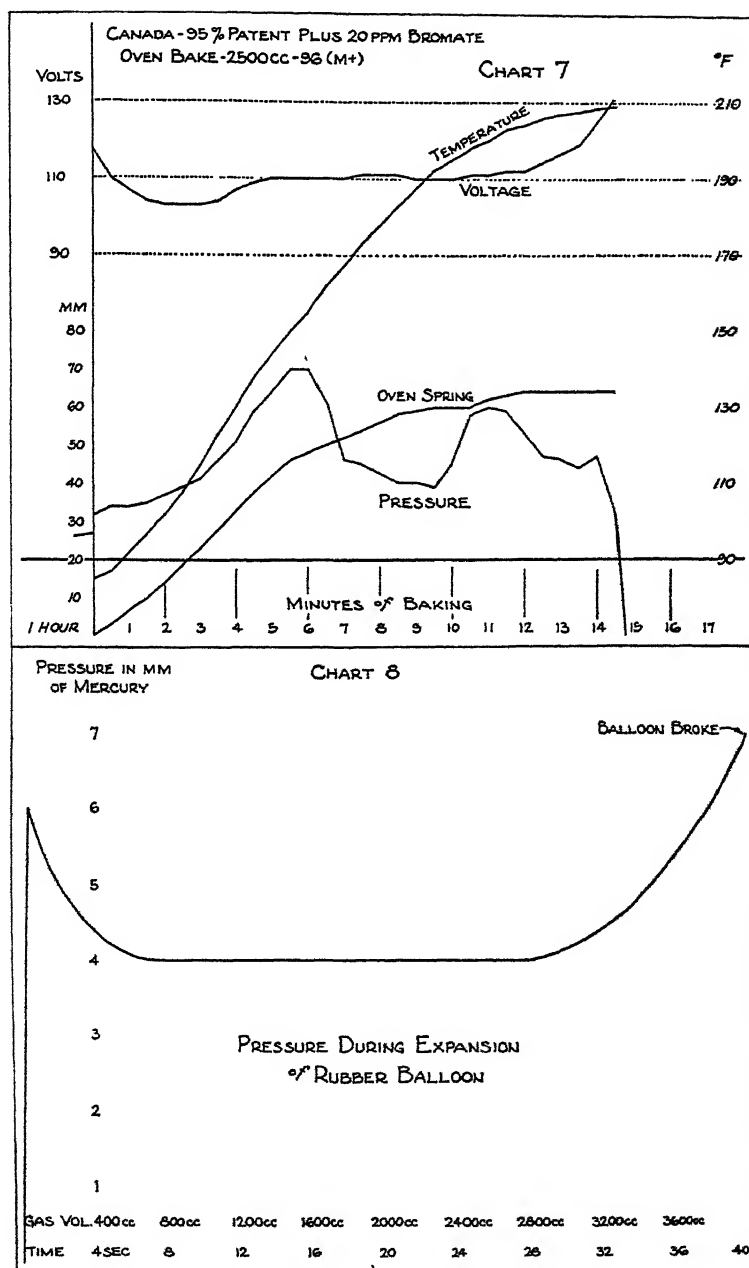
Crust bread baked from this same dough shows very little oven spring, a very green coarse texture, and all the characteristics of an untreated, unoxidized dough.

Chart 7 shows the same dough after being treated with bromate and is one that gives a large loaf of bread with good texture and maturity. It is to be noted here that the pressure during proofing is somewhat higher than with the untreated dough and that the pressure at the onset of baking rose at a steeper rate, reached a much higher level, and at the completion of the starch swelling showed only a partial drop in pressure. It also gives a subsequent rise upon the generation of alcohol pressure, which is followed by a decrease toward the end of the baking.

The outstanding characteristics of these pressure curves are:

1. The level at which they start.
2. The steepness of the ascending slope and height they reach and the length of time during which the pressure persists.
3. The temperature at which the pressure first falls.
4. The amount of the pressure fall after failure and its duration.
5. The amount of its return during alcohol distillation.

To aid in the interpretation of these curves Chart 8 shows the pressure in a thin elongated rubber balloon as it is blown up to the bursting point. It is to be noted that after the inflation is well started



the pressure remains constant, although the strain in the balloon is steadily becoming greater. Also as the breaking point is approached there is a marked rise in pressure until the tensile strength of the rubber is reached and the balloon bursts.<sup>3</sup> Calculations indicate that where the modulus is constant, a rubber balloon should expand with little change in pressure. When the pressure rises the modulus is increasing and the rubber approaches its elastic limit. One can interpret the pressure results obtained in our baking work in a similar manner; that zone where little pressure rise occurs suggests a constant modulus in the dough. Where the pressure rises the modulus may be considered as increasing and the cell walls approaching their elastic limit.

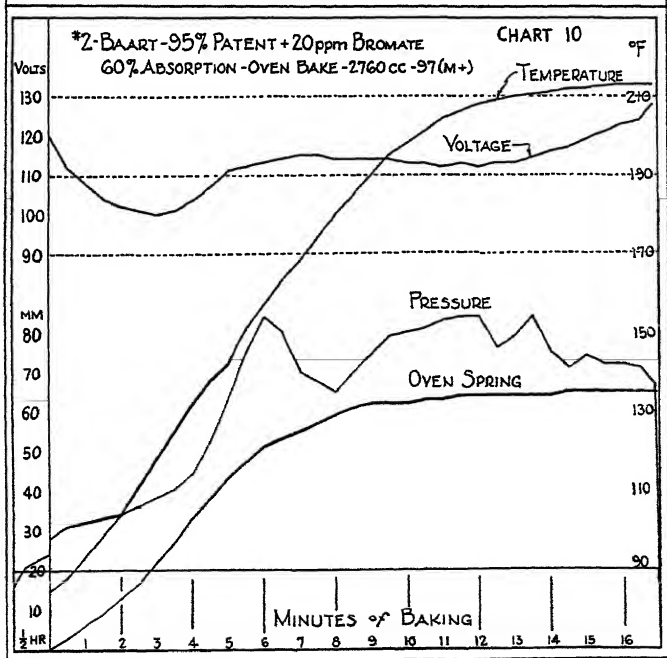
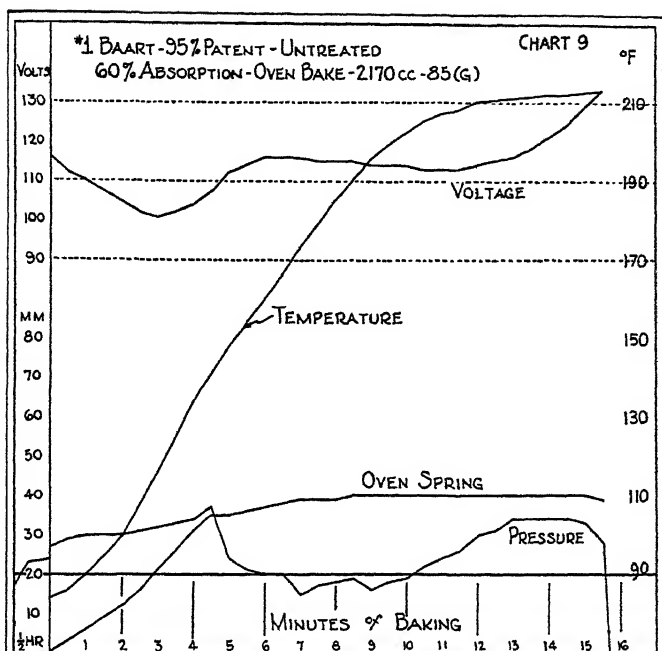
Our records show that where the best texture is obtained in bread a sharply ascending pressure curve is obtained as the dough is heated. Where little pressure rise occurs, good bread is not obtained. This zone, up to the peak, can be termed the "texture-creating zone" of the oven spring. If the pressure drops low after this peak, the corresponding bread exhibits a texture with non-uniform, round, enlarged bubbles, whereas if the pressure is well maintained during the heating and bridged over to the alcohol zone without much collapse, fine, elongated cells with matured texture are observed. This type of curve with steep ascent and no collapse is the most desired and exhibits the form for a good bread-making dough. Collapse is suggestive of puncture and possible coalescence of bubbles and a loss of texture.

A characteristic of our results where a drop of pressure occurs during or after starch swelling is that the oven spring shows no corresponding fall. In fact, in all cases the volume of the test dough is maintained and usually a slight increase in oven spring continues. One might find it difficult to explain how this can be. Obviously if the volume continues its rise there can be no complete loss of gas from the loaf during this fall in pressure. The explanation lies in the relation of bubble surface to gas volume. When two gas bubbles coalesce and form one, their combined surfaces form the new bubble and as the same amount of gas requires less surface in one bubble than in two the strain on the film must decrease, and this releases the stress and hence the pressure falls. It is believed that this is the phenomenon occurring when any marked drop in pressure occurs and hence a pressure drop indicates loss in texture by breaking and probable coalescence of bubbles but not necessarily excessive loss of gas from the loaf itself.

In these experiments the outer surface of the dough was prevented from controlling the pressure in the loaf by being sprayed with a dilute

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<sup>3</sup> A circular balloon gives a similar curve but lacks the period of constancy.





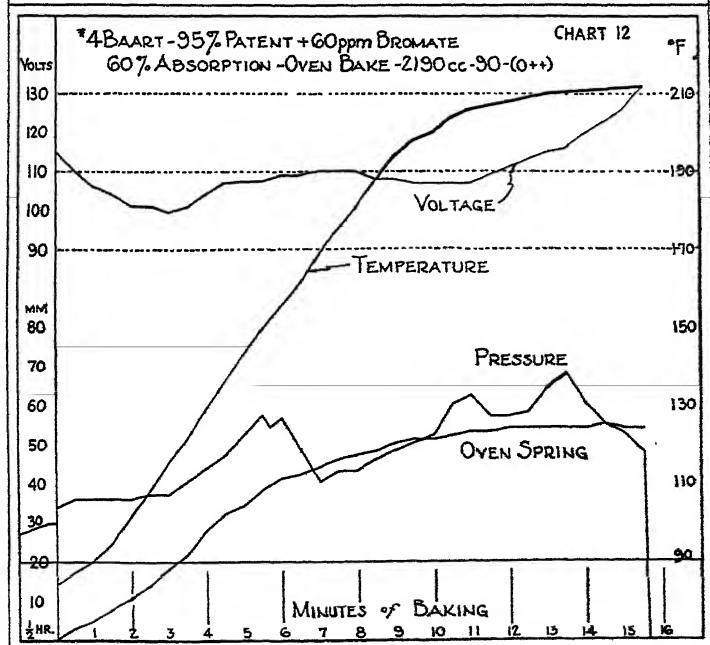
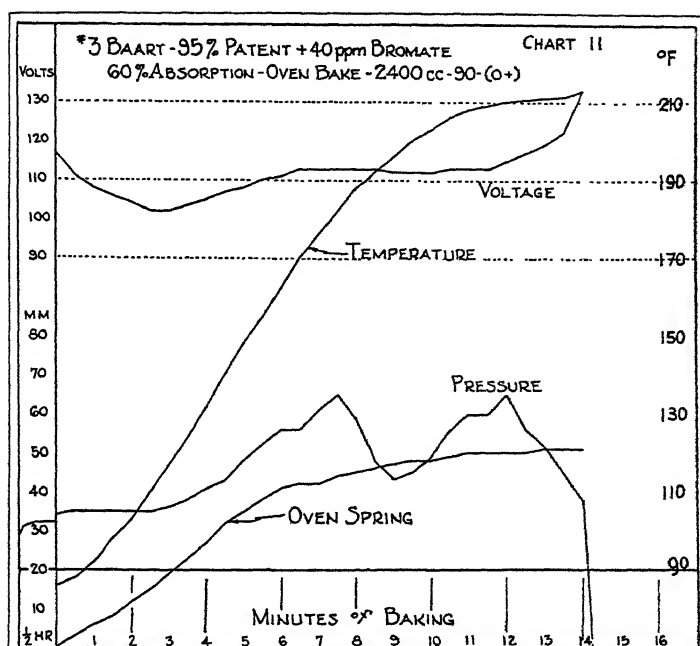
salt solution containing glycerin, and also by avoidance of water loss through use of a covering over the top of the pan.

We have been unable from the pressure curves alone to explain the phenomena that occur in overoxidized doughs, the characteristics of which may be tougher crumb, collapsed cell structure, occasional knotty whorls, a drawing in of the sides or the bottom of the loaf, and a parting of the crumb inside the loaf, usually under the crust. All of these phenomena indicate greater forces drawing the loaf inward. It may be that the voltage curves give the clew to this phenomenon and indicate a change in the dough properties with different oxidation. Gluten or starch may become firmer or more contractile in nature; hence a shrinkage occurs which gives its characteristics to overoxidized bread.

The work reported in Charts 9 to 12, showing the variations in dough properties during baking when treated with varying amounts of potassium bromate, has been repeated on different kinds of flour, with similar results.

### Discussion

These charts offer numerous suggestions as to the relation of physical properties and their influence upon dough while baking. It is apparent that all doughs show a voltage rise during approximately the same temperature range, which coincides with the swelling of starch. It is also apparent that the pressure in the dough shows a marked rise during the same period. The most rapid portion of the oven spring is also completed by the time this voltage rise has occurred. These generalizations can be seen in all of our work. It is apparent that the swelling of the starch by the taking up of water has reduced the conductivity and at the same time increased the force required to stretch the dough. The swelling of starch must result in the transfer of water from the gluten to the starch, resulting in a tightening of the gluten strands with a resulting decrease in their fluidity and hence an increase in their tensile strength. At the same time, the swelling of the starch must fix the location of the starch granules in the dough mass so that little further rearrangement of these granules may occur to enable the films to become thinner. There must hence be an increased demand on the elastic extension of the gluten for further oven spring, and starch rearrangement must be accomplished with increasing difficulty. When the pressure reaches that point where the tensile strength of the bubbles is exceeded and they puncture or break, with a resulting drop in pressure in the dough, the rate of oven spring decreases and more slowly exhibits itself thereafter. Hence, the earlier this drop in pressure occurs the lower the oven spring and the smaller



the volume. At the same time, if the breaking of the bubbles should occur early in the baking process, before the plastic properties of the dough have been largely lost by the swelling effect of the starch there must result greater coalescence of the bubbles from this puncturing of cells. Hence, the earlier the pressure drop occurs and the greater its magnitude, the coarser the texture one should expect. Our results indicate that this is largely true. The characteristics of the dough as shown by the dropping of the plunger (Chart 5) also indicate that the dough is at its maximum fluidity prior to and during the early stages of starch swelling. This is further evidence that the dough is in the optimum condition for coalescence of bubbles at this stage, so that if the cohesive properties of the bubbles are not strong enough to support the pressure, coalescence will occur.

Our work indicates that one of the effects of potassium bromate upon dough is to increase the pressure required to expand the dough, suggesting that the modulus of elasticity has been raised by the oxidation. The higher the degree of oxidation, the higher this initial pressure becomes, both at the end of the proof and at the beginning of baking. Pressure curves showing the sharpest rise and the most continuous maintenance of the pressure during all stages of baking without any failure or drop are found in those cases where the finest texture is obtained. Such doughs are rather rare, and ideal conditions must be obtained in order to get such a pressure result. However, when such a dough is obtained the resulting tinned bread is of very fine texture, large volume, and thin crust.

Halton and Scott Blair (1937) have pointed out that the quality of dough largely depends upon the relationship of the modulus of elasticity to the viscosity of the dough. In an effort to interpret our results in terms of their findings we have considered the following dough properties:

#### A. *Elastic Properties*

1. The force required to stretch the dough film a unit distance or modulus of elasticity.
2. The elastic extensibility of the dough.
3. The increase in modulus of elasticity.
4. The tensile strength of the dough.

#### B. *Plastic Properties*—The plastic flow of the dough includes

1. The rearrangement of the structural position of the starch granules in the dough films.
2. The flow of gluten itself.

It is obvious that the larger the number of bubbles a dough contains, the thinner the dough film becomes and the greater the amount of film surface present in the dough. It would appear that the thinness of a dough film should ultimately be limited by the diameter of the larger

starch granules in the dough. Preliminary microscopic observations on this point in our laboratory support this idea. If the diameter of the large starch granules determines the minimum film thickness that can be obtained, then the elastic properties come fully into play when the starch rearrangement has gone as far as it can in a particular dough. Starch rearrangement results from elastic strain which can thereby be relieved to some degree.

It is probable that many doughs are not sufficiently strong to carry the starch rearrangement to its ultimate limit before the film is broken. This must be the case in the ordinary unoxidized doughs from freshly milled flour. The pressure curves in such a dough during proofing and during the early stages of baking show little rise though they give the usual pressure jump on entering the oven. In such doughs large amounts of plastic and elastic extension must occur with a low modulus of elasticity. However, the tensile strength is low and is quickly reached in oven spring so that much rupture and coalescence occur. It would appear obvious that for doughs to exhibit their elastic phenomena, plastic flow must ultimately be subdued and become secondary. This suggests that primary plastic flow in doughs may be starch rearrangement, which by its motion interferes with the full elastic forces in the dough becoming aligned in the direction of the strains in the bubble surfaces. These elastic forces can only exhibit themselves to their maximum degree when starch rearrangement approaches its ultimate limit.

If a film of dough can no longer become thinner, then the pressure in a dough bubble must rise until the breaking point is reached. This is the phenomenon we have at the peak of the pressure curves. One might say the higher the pressure rises the greater the elastic modulus and the tensile strength. Given these properties, the higher the oven spring rises the greater must be the elastic extensibility and starch rearrangement rather than the plastic flow of the gluten. This would suggest that these are the physical properties which determine dough quality as evidenced by oven spring and fine texture. The greater their magnitude and duration, the better the texture and volume of the bread. However, should not plastic flow finally relieve the strain, there would be a shrinkage in a loaf with the final drop of pressure.

Stress would appear to be uniformly distributed throughout a dough mass, since any inequalities would be adjusted by the yielding of the films to maintain the balance of forces necessary in such an extended delicate foam structure.

During the period after the tensile strength has been exceeded (shown by drop of pressure on the curves) unequal bubble sizes will

be produced by rupture occurring at the weakest points in the films, thus producing larger cells with relief of stress and strain in the entire structure until generated or expanding gas renews the forces. It is of particular interest that the alcohol pressure usually does not greatly exceed the maximum carbon dioxide pressure. This top pressure is determined by the tensile strength and thus may be approximately the same in the two zones.

It can very well be that the alcohol pressure can produce further disruption of the cellular structure but it is very unlikely that cell formation or any coalescence will occur during this period because such alcohol pressures occur after the dough is set and substantially no longer extensible. It is very possible that elongated cell structure can be produced by alcohol pressure, though elongated cell structure is more likely to be produced by the motion of the dough during the oven spring in a dough which does not coalesce or lose its cellular structure but carries the pressure originally generated by carbon dioxide through to the alcohol zone and final coagulation.

In crust bread, because of unequal heating, the alcohol pressure in the exterior portions of the loaf must balance itself against the carbon-dioxide pressure in the middle. Any cell structure which has been disrupted by the carbon-dioxide pressure, in the outer layers, and hence unable to retain the subsequent alcohol pressure, will be packed against the crust by interior pressure in the loaf. The relation of this to crust thickness and texture gradation will be immediately seen.

### Summary

A method of testing doughs described by Baker has been investigated.

The crustless bread thus produced enables one to study the properties of dough independently of the influence of crust, while baking.

Dough is made the resistance unit between electrodes carrying alternating current. The voltage required to keep a constant wattage flowing, the temperature rise thus produced, the rate of oven spring, the fall of a bob through the dough, and the pressure generated in the dough during the baking, have been studied.

The rate of temperature rise in dough is retarded by carbon dioxide and alcohol being driven out of solution. These gases are the source of the pressure developed in baking dough.

The properties of the dough change most markedly while baking during the period of starch swelling, there being a marked rise in voltage, a rise in pressure within the dough, a marked change of the rate at which the weight falls through the dough, and a marked change in the rate of oven spring during this period.

The pressures generated within dough while baking are determined by the physical properties of the dough and indicate many of the characters of the bread which will be made from the dough.

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### COMPARATIVE DATA OBTAINED ON SOME 1938 HARD RED SPRING WHEAT VARIETIES BY THE USE OF FOUR BAKING FORMULAS

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The literature dealing with applications of various test baking methods or formulas to experimental flour testing is for the most part familiar to cereal chemists. Variations in mixing and fermentation times, as well as in the number and quantities of ingredients included in the baking formula, have been extensively investigated.

Blish (1934) pointed out that the basic procedure should be used essentially as a point of reference. Other supplementary methods could then be imposed, and the results obtained by the different procedures reported for comparative purposes.

Herman and Hart (1927) made a comparative study of different methods and formulas, and showed that powdered milk added to the basic formula decreased the loaf volume, crumb color, and grain, while it improved crust color. The absorption was not increased when milk powder was added. A shortening agent was incorporated with the basic formula in another experiment. Crumb color, grain, and crust color were improved by the shortening (a hydrostearoleine was used). Loaf volume was decreased when 3% of this shortening was employed.

Grewe (1928) found that dried skimmilk improved the baking quality of hard red spring wheat flour to a greater extent than hard winter wheat flour. In other cases the baking quality was impaired by milk unless flour improvers were also used. The color of the crumb was rendered more creamy and glossy by dry skimmilk, while break and shred were also improved. Skovholt and Bailey (1931) cited the growing importance of dried skimmilk in the baking industry, owing chiefly to the increased popular demand for greater nutritive value in the daily diet. These workers found that dried milk, especially if preheated, substantially improved the baking quality of a strong hard-wheat patent flour.

Aitken and Geddes (1934) concluded that the malt-phosphate-bromate formula with three-hour fermentation time yielded maximum differentiation between samples and gave the largest absolute loaf volumes when 10 composite samples of western Canadian hard red spring wheat flour were baked by various methods. Harris and Sanderson (1938) showed that the malt-phosphate-bromate formula gave better differentiation than the standard basic procedure between samples of North Dakota hard red spring wheat flours. A higher positive correlation was obtained between flour protein and loaf volume by the malt-phosphate-bromate method, while larger loaves were also produced, as compared with the standard basic procedure.

In view of the rather meager amount of experimental work which has been published regarding the effects of shortening and dried skimmilk as compared with the results obtained with the standard basic and malt-phosphate-bromate methods, it was thought desirable to obtain further information relating to the effect of formulas containing these ingredients upon North Dakota hard red spring wheats. Dried milk and shortening are quite commonly used in commercial baking, and the need of obtaining data on hard red spring wheat flour by the use of experimental formulas containing these ingredients has often been suggested to the author. Four methods or formulas were accordingly chosen for use in the following comparative studies.

The determination of the baking quality of a series of wheats which differ in genetic origin is always more difficult than in the instance of wheats which have a more or less common origin. The diversity of variety introduces quality differences into the picture. When dealing with plot samples the chemist is usually working with material at a fairly uniform protein level, and relationships between protein content and baking strength are more difficult to bring out than where a wide range in wheat protein is encountered. The latter situation usually arises when samples are drawn from a large area in which relatively few varieties are grown. Crop survey work would

fall into this classification. The investigational material used in this study would belong in the first group.

### Material and Methods

The wheats included in the present investigation consisted of varieties and selections which differed in genetic origin, but all belonged to the hard red spring wheat class. The samples were quite free from damage and no complicating factor was introduced because of injury from any cause. The samples, 26 in number, were cleaned, scoured, and tempered to a moisture content of 15% previous to milling into straight-grade flour on an Allis-Chalmers experimental mill. The protein content of the wheat was determined, as well as the flour protein. The flours were then baked by four methods—the standard basic; the malt-phosphate-bromate, which contained 0.3% of diastatic malt, 0.1% of ammonium di-hydrogen phosphate, and 0.001%  $\text{KBrO}_3$ ; a milk-shortening formula which contained 4% dried skimmilk and 3% shortening in the form of highly refined lard; and the milk-shortening formula plus 0.001%  $\text{KBrO}_3$  in addition to the basic ingredients. All formulas contained 5% sucrose. The mixing time used was two minutes in the Hobart-Swanson. Fermentation time and temperatures were as recommended in the standard basic method. The same absorption was used for each baking method.

### Discussion

The grades, test weight per bushel, crude wheat and flour protein, and flour ash are shown in Table I. It will be noticed that the grades and test weights were all relatively high, only one sample grading lower than 1 Dark Northern Spring, while the lowest test weight was 57.4. The wheat protein ranged from 13.3% to 17.0%, a difference of 3.7%, while the flour protein ranged from 12.5% to 16.2%, a difference also of 3.7%. These wheats showed a substantial variation in protein content and should, therefore, be expected to differ in baking quality because of these differences. Substantial differences in flour ash were also evident among the wheats.

The loaf volumes, texture, and color scores and corresponding means are presented in Table II, the data being grouped according to loaf volume, texture, and color, rather than method, to facilitate baking method comparisons. It is evident that the mean loaf volume increases in going from the standard basic through the milk-shortening and milk-shortening-bromate to the malt-phosphate-bromate method. The texture and color scores are not so easily differentiated according to baking methods used, and accordingly recourse must be had to statistical methods of handling the data to bring out these relation-



TABLE I

COMPARATIVE GRADES AND ANALYTICAL DATA OF SAMPLES ARRANGED IN ORDER OF INCREASING WHEAT PROTEIN CONTENT

Lab. No.	Grade <sup>1</sup>	Test weight, lbs. per bushel	Crude protein <sup>2</sup> N × 5.7		Ash <sup>2</sup>
			Wheat	Flour	
			%	%	%
38-9-38	2 DNS	57.4	13.3	12.5	0.51
41	1 HDNS	61.0	13.4	12.5	0.48
40	1 HDNS	60.6	13.5	12.6	0.54
36	1 HDNS	62.0	13.6	13.1	0.54
47	1 DNS	59.5	13.7	12.6	0.45
39	1 HDNS	60.7	13.8	12.7	0.54
49	1 HDNS	61.3	13.8	13.0	0.65
28	1 DNS	58.6	13.9	13.4	0.47
42	1 DNS	59.5	13.9	12.5	0.50
45	1 HDNS	60.8	14.1	13.1	0.54
31	1 DNS	59.6	14.2	13.4	0.53
32	1 HDNS	60.4	14.2	13.5	0.61
46	1 HDNS	60.3	14.2	13.2	0.52
43	1 DNS	59.3	14.3	13.0	0.53
48	1 HDNS	62.1	14.3	13.1	0.50
30	1 HDNS	60.6	14.4	13.6	0.52
34	1 HDNS	60.8	14.4	13.5	0.39
50	1 HDNS	61.5	14.4	13.3	0.51
37	1 HDNS	62.0	14.5	12.6	0.58
44	1 HDNS	61.2	14.5	14.0	0.52
52	1 HDNS	62.5	14.5	13.5	0.68
29	1 HDNS	62.0	14.6	13.4	0.54
35	1 HDNS	60.6	14.8	13.5	0.37
51	1 HDNS	62.0	14.8	13.5	0.41
33	1 HDNS	60.4	15.4	14.5	0.41
53	1 HDNS	60.0	17.0	16.2	0.42

<sup>1</sup> Unofficial.<sup>2</sup> 13.5% moisture basis.

ships. The means, standard deviations, coefficient of variability, and simple correlation coefficients calculated from the analytical data are shown in Table III.

Examining first the values for the means and standard deviations it is seen that flour protein averages 1.0% below wheat protein, and is slightly more variable than the latter. The ash content has the highest relative variability of any of the values listed, due, to some extent at least, to the small quantity of ash contained in the flour as contrasted to the larger values of the other variables. The correlation constant shows a high relationship between wheat and flour protein. The constants between flour protein and loaf volume are all significant with the exception of the one between protein and milk-shortening-bromate loaf volume. The malt-phosphate-bromate value is small but significant. It will be noticed that the highest correlation exists between flour protein and basic loaf volume, which is contrary to the

TABLE II

COMPARATIVE RESULTS OBTAINED BY THE FOUR BAKING METHODS ARRANGED IN ORDER OF INCREASING WHEAT PROTEIN CONTENT

Lab. No.	Loaf volume				Texture				Color			
	Stand- ard basic	Milk- short- ening	Milk- short- ening- bromate	Malt- phos- phate- bromate	Stand- ard basic	Milk- short- ening	Milk- short- ening- bromate	Malt- phos- phate- bromate	Stand- ard basic	Milk- short- ening	Milk- short- ening- bromate	Malt- phos- phate- bromate
38-9-38	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
41	477	598	653	650	94.0	94.5	93.5	94.0	93.0	94.0	94.0	93.0
40	495	544	649	720	94.0	93.5	93.0	90.0	94.0	94.0	93.5	95.0
36	490	564	601	697	94.5	93.5	94.0	91.0	93.0	93.0	94.0	94.0
47	541	567	611	666	94.0	94.5	94.0	93.0	93.5	94.0	94.5	93.5
47	491	580	619	710	93.0	93.5	95.0	92.5	93.0	92.0	94.0	93.0
39	467	573	631	710	93.5	93.5	93.5	93.0	93.0	93.0	93.5	93.0
49	520	548	609	674	93.5	94.0	94.0	92.0	92.0	93.0	93.0	93.0
28	595	618	688	762	93.0	93.5	92.0	92.0	93.0	93.0	94.0	93.0
42	490	594	594	620	93.5	93.5	94.5	92.0	93.0	93.5	94.0	92.0
45	489	568	616	693	93.5	93.5	93.5	90.0	93.5	93.5	94.0	94.0
31	522	548	617	732	93.5	94.0	92.0	92.0	93.0	94.0	93.5	93.5
32	506	544	602	713	94.0	94.5	93.0	92.0	93.0	94.5	94.0	93.5
46	548	568	589	630	93.0	94.0	94.0	90.0	94.0	94.0	94.0	95.0
43	488	596	640	644	93.5	93.0	92.0	91.5	92.0	93.5	94.0	93.0
48	572	621	686	738	94.0	93.5	92.0	90.0	94.0	94.0	93.0	95.0
30	532	578	632	715	92.0	93.5	92.5	91.5	92.0	93.0	93.5	93.0
34	600	601	641	707	92.0	93.5	91.5	90.5	93.0	93.5	93.0	92.5
50	560	610	717	707	93.5	94.0	92.5	91.0	94.0	94.0	93.0	92.0
37	546	589	660	683	93.0	94.5	92.5	93.5	94.0	94.0	94.0	93.5
44	582	616	644	718	93.5	93.5	92.5	92.0	94.5	94.0	94.0	94.0
52	570	612	662	701	94.0	93.0	91.0	91.0	94.0	95.0	93.5	94.5
29	660	626	761	784	92.5	94.0	92.0	92.0	94.0	94.0	94.5	93.0
35	535	610	672	689	93.5	93.0	91.5	91.0	94.0	94.0	94.0	94.0
51	540	622	653	699	93.0	94.5	92.5	92.0	93.5	94.0	94.0	94.5
53	535	568	591	706	93.0	93.5	92.5	92.5	93.5	94.0	93.5	93.0
53	693	753	737	764	92.0	91.5	90.0	91.0	94.0	95.0	93.0	94.0
Means	540.15	592.92	645.19	701.42	92.33	93.65	92.75	91.65	93.38	93.75	93.69	93.50

TABLE III

TABLE OF STATISTICAL CONSTANTS  
MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIABILITY

	Means $\bar{x}$	Standard deviations	Coefficients of variability
Wheat protein $\bar{x}_c$	14.30	0.719	5.03
Flour protein $\bar{x}_c$	13.30	0.752	5.65
Flour ash $\bar{x}_c$	0.502	0.114	22.71
Simple Correlation Coefficients			
Variables correlated			
X	Y	rx	p <sup>1</sup>
Flour protein	Wheat protein	+ .9148	<.0001
Flour protein	Loaf volume (standard basic)	+ .7158	<.0001
Flour protein	Loaf volume (milk-shortening)	+ .6869	.0002
Flour protein	Loaf volume (milk-shortening-bromate)	+ .3650	.0672
Flour protein	Loaf volume (malt-phosphate-bromate)	+ .4635	.0172

<sup>1</sup> p = probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.

results usually obtained, as the malt-phosphate-bromate method commonly gives the highest correlation between these variables. There is a possibility in the present instance that the diversity of genetic origin and variety has introduced a complicating factor into the situation and has altered the degree of relationship between flour protein and loaf volume as shown by the different methods.

In order to evaluate the significance of the differences in the results between baking methods, as well as between wheat varieties, the analysis of variance was applied to the data. Two criteria of classification were used—grouping according to baking method and according to wheat variety. The mean square for interaction can be used to test the variety means only if an assumption is made that there is a random selection of methods. The inclusion of the color and texture scores in the analysis may be somewhat debatable owing to the slight variability evident among the percentages and the fact that they are subjective measurements. The results obtained are set forth in Table IV, which shows the source of variation with corresponding degrees of freedom, sums of squares, mean squares, F values, and the 5% point.

TABLE IV  
ANALYSIS OF THE DATA

Source of variation	De- grees of freedom	Sum of squares			Mean square			F values			5% Point
		Loaf volume	Tex- ture	Color	Loaf volume	Tex- ture	Color	Loaf volume	Tex- ture	Color	
Total	103	579,746	145.04	50.06	—	—	—	—	—	—	—
Between methods	3	373,696	60.17	2.24	124,565.33	20.0567	0.7647	166.83	33.37	2.18	2.73
Between varieties	25	150,051	39.79	22.12	6,002.04	1.5916	0.8948	8.04	2.65	2.53	1.66
Interaction (Method X Varieties)	75	55,999	45.08	25.70	746.65	0.6011	0.3427	—	—	—	—

Variations in mean loaf volumes were significant as between baking methods and also between varieties. The same tendency appeared with reference to variations in texture. With respect to color, variations between methods were not significant, but were significant between varieties.

Break, shred, and the general appearance of the loaves were substantially enhanced by the addition of dry skim milk and shortening.

### Summary and Conclusions

A series of 26 samples of hard red spring wheats differing in genetic origin were milled into straight flours on an Allis-Chalmers experimental mill. The wheats were graded and analyzed for crude protein. The test weights and grades of these samples were relatively high.

The resultant flours were analyzed for crude protein and ash, and were baked by four procedures—the standard basic method with 5% sucrose; a milk-shortening formula, including 4% dry skim milk and 3% shortening; a milk-shortening-bromate formula, which included the milk-shortening formula plus 0.001%  $\text{KBrO}_3$ ; and the malt-phosphate-bromate formula. The three latter formulas were all superimposed upon the standard basic.

The results obtained from the analytical work showed a range of 3.7% in both wheat and flour protein between the highest and lowest protein samples. The ash was quite variable in the flours of this series. A very significant difference in mean loaf volume among the four baking methods was revealed by the analysis of variance. These mean loaf volumes increased in the order in which they are given. The mean texture scores also differed significantly between the methods, while crumb color showed no significant differences.

If a random selection of methods were assumed it was evident that there were significant differences among variety means in respect to loaf volume and color and texture scores, thus demonstrating significant variability in baking strength among the hard red spring wheat varieties.

Correlation coefficients for flour protein and loaf volume for the four baking methods were +.7158, +.6869, +.3650, and +.4635, respectively. All the correlations except the third one were significant. These values would doubtless have been definitely larger if a wider range of protein content and baking strength had been present in the series of wheats studied.

The data obtained in the present study demonstrated the differences in loaf volume, and to a lesser degree in texture score, yielded by different formulas used in baking flours milled from hard red spring wheats which differ in genetic origin. Current opinions regarding differences in the baking strengths of the individual wheats investigated were also substantiated by this work.

#### Acknowledgments

The author is indebted to T. Sanderson, who conducted the experimental milling tests, and to Muriel Johnson, who performed the statistical work connected with this study. Acknowledgment is also made of valuable advice regarding the statistical analysis of the data contributed by Gertrude M. Cox, Statistical Laboratory, Iowa State College.

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## PRESIDENTIAL MESSAGE

W. F. GEDDES

University of Minnesota  
Saint Paul, Minnesota

(Read at the Annual Meeting, May 1939)

The history of our Association records that on the eighth day of May, 1915, eleven mill chemists in the Southwest met in Kansas City, Missouri, at the call of the late Mr. H. E. Weaver, to form an organization for "the advancement of the science as applied to cereal analysis." It is particularly fitting that we should hold this, our twenty-fifth annual meeting, in the city in which our Association was formed and thereby acknowledge the debt we owe to our charter members, all cereal chemists of the Southwest, in establishing our organization on a firm scientific foundation.

It seems only appropriate that we should pause briefly and take stock of some of the developments of the past twenty-five years. A retrospect alone affords a proper comprehension of our present status and leads us to the conviction that we live in a state of continuous transition, that our ideas of today are merely the precursors of others and that even they cannot for any length of time satisfy the requirements of science. Man aims to achieve, the human spirit strives to surmount difficulties; it glories in creative power, and the illustrious achievements that have been recorded in the application of chemistry to industry during the past quarter of a century have made a striking appeal to the human mind.

In primary and secondary industry, it has meant the creation of increased wealth, increased employment and enhancement of the general welfare of the people. For these reasons, science is considered today one of the most effective agencies in influencing the attitudes of thoughtful men.

Progress has been so rapid that it is difficult, indeed, for us to appreciate the conditions that prevailed in our own specialized field little more than twenty-five years ago. Prior to 1915, our history records that in mills the character of the mill mix was governed by the manager's "wheat sense," the price policy, and the weather. Flour bleaching was limited to the use of nitrogen peroxide, wheat was chewed to determine its quality, washed glutens were standard determinations among millers and flour buyers, and the miller's wife was the "test-baker" whose verdict decided the quality of the flour. Cereal chemistry was just beginning to emerge as a distinct branch of the young and flourishing science of biochemistry, and a few of the more progressive

millers were coming to realize that laboratory control was essential. With the advent of the laboratory much confusion in the trade resulted, since the methods of conducting and reporting analyses varied and secrecy was the order of the day.

Thus the Association had a simple and natural origin, and therein we find its primary aim, the establishment, by means of research and discussion, of standard methods of procedure in the analysis of cereal products. From this modest beginning as regards both membership and activities we have experienced a healthy growth. At the present time our total membership stands at 608, 69 of whom reside in foreign countries, and the majority of the leaders in cereal chemistry throughout the world, whether in university work, research, or industry, are now banded together in this body to serve the well-being of matters relating to applications of their specialized knowledge. One of the most noteworthy facts is the manner whereby our Association has unified cereal chemistry in this country from coast to coast. This Association has brought together the cereal chemists from many laboratories, and we have all grown in breadth of vision and in increased usefulness in our profession because of the contacts and friendships which have developed in this Association. Furthermore, we have attained a professional status in the cereal industry which would have been impossible to secure had we all insisted on following our own individual pathways. A highway of cereal chemistry now exists where 25 years ago there were only overgrown footpaths through a wilderness.

Our objectives have been expanded to include not only the study and development of standard methods but also the advancement of scientific and technical research in the cereal and associated industries, promotion of the spirit of scientific cooperation, "the encouragement of more general recognition of the importance of the chemist and biologist in the cereal industries and the maintenance of high professional standards as conditions for membership."

The extent to which these objectives have been achieved is to be detailed elsewhere at this meeting. The chemistry of flour milling technology has advanced rapidly from the organic chemical to the biochemical and physical viewpoints and techniques, and today the industrial laboratory has been placed on a plane where it takes part in original discoveries as well as in their applications. The fertility of research and the wealth of literature has been so great that it would have been virtually impossible for many cereal chemists, who are without extensive library facilities, to keep in touch with recent advances without the existence of our journal, *Cereal Chemistry*, which is attracting an ever increasing number of papers on fundamental and technological researches in this field.

The success which has been attained by our various technical committees illustrates the value of the collaborative method of attack; progress in technological fields requires the coordinated effort of many individuals, each fitting his contribution into its place in the puzzle.

An accounting of the activities of the current Association year will be given in the report of the Executive Committee. It has been a successful one scientifically and financially. The policy of effecting a greater continuity in the membership of our technical committees has been developed further; this has only been made possible through the willingness of several members to carry on their tasks, and to these we are deeply grateful. This year a new committee—the Committee on Cooperative Research—was appointed to cement more closely our relations with technical organizations in the milling and baking trades. This committee has cooperated with the American Society of Bakery Engineers in undertaking an extensive study of the effect of the mineral constituents of water on dough fermentation, the results of which were presented at the annual meeting of the Bakery Engineers held in Chicago last March.

The real work of this Association is carried out by the local sections, the various committees, the other officers, and the staff of *Cereal Chemistry*. A spirit of friendliness and cooperation has prevailed at all times, and I am deeply grateful to all for this attitude which has made the duties of the president so inarduous and pleasant.

At the business session you will be asked to vote on certain proposed amendments to the constitution which have crystallized as the result of very careful consideration extending over the past three years. It is not proposed here to discuss those amendments designed to facilitate and regularize our business activities, but it seems desirable that I should comment briefly on the proposal to strengthen our membership requirements. This is a matter upon which I fully realize there is room for difference of opinion. On the one hand we may take the attitude that this Association can perform the greatest service to society by maintaining our present membership requirements which admit those of rather limited formal chemical training or practical experience as such individuals would be particularly benefited through membership. On the other hand, one of the avowed purposes of this Association is the maintenance of high professional standards as a condition of membership and many feel that our existing requirements are not sufficiently rigid to ensure our continued recognition as a strictly professional organization.

The lawyer and the doctor, by a sufficiently long, arduous, and specialized course of learning, become sufficiently acquainted with



their specialties to be admitted by the State to practice. The general public knows the purpose of these professions and recognizes that those practising them are reasonably competent to do so. The public cannot for itself judge the value of the chemist, for it knows but little of his work or of the standards which should stamp him as a sound member of his profession.

It is worthy of note that steps have been taken by the American Chemical Society to prepare an accredited list of colleges offering specialization in chemistry, the curricula of which are regarded as of a sufficiently high standard to designate their graduates as adequately trained chemists. The News Edition of *Industrial and Engineering Chemistry* for April 20th, carries an interesting case history of a decision of the United States Supreme Court in which the professional status of the chemist is legally established. The trend of the times in all fields of science is towards more rigid requirements, and we would be well advised to take cognizance of these developments.

Until such time, if ever, as it should seem feasible and desirable to a large majority of the chemists of this country to exert the same sort of legal restriction for the practice of our profession as is now clearly desirable for law and medicine, it is my humble opinion that this Association can best carry out its aims and objects by insisting on sufficiently high standards of admission as to stamp its members as sound and reliable chemists who can safely be employed and relied upon for any work within their special fields. We have maintained high standards by bringing within our membership the majority of the outstanding chemists in the field, and it appears that hereafter we should set such standards of admission as will ensure that membership be not too easy to attain and be, therefore, valuable of attainment by the younger chemist. What is gained too easily is little appreciated. Moreover, chemistry is more and more assuming cultural and social values and in the future most educated persons may be expected to possess, as a kind of common knowledge, much of that which is now viewed as highly specialized information.

I look forward to the day when membership in the American Association of Cereal Chemists will be an almost necessary qualification for the consideration of an individual for the post of director of a cereal laboratory or for the more important technical positions in such laboratories.

To be a chemist requires training, and while a degree in chemistry is the best indication that this has been successfully accomplished, it would be a narrow view to take that all standards of admission should be referred to a purely university conception of education. The

amendments being brought forward by the Membership Requirements Committee provide that four years of practical experience involving varied chemical and technological determinations will be recognized in lieu of two years of formal training. It is also proposed to establish a Membership Application Committee to pass upon all applications, thereby relieving the Treasurer and the Executive Committee of the duty of reviewing applications. In the event the Association agrees that this is a desirable method of dealing with applications, I would recommend that the personnel of the committee should be so selected in successive years as to ensure that continuity of opinion which is essential for the creation of a reasonable permanence of standard.

It is the prerogative of the president to make suggestions as to possible future activities. One of our aims as expressed in the constitution is the encouragement of "a more general recognition of the chemist and biologist as essential factors in the development of the cereal industries." In this connection our Publicity and Inter-relations Committees have done excellent work, but it seems to me that there is still another avenue of approach to the public which we can legitimately explore. Can we not establish annual public lectures, given under the auspices of our local sections at the various centers, which would be stimulating and informative alike to the general public and to those connected with the cereal industries? These might well deal not only with the general advances which are being made in cereal technology, but also with the part that our own members have played. In fostering such an activity, however, we must bear in mind that the essence of our success will depend less on publicity for the organization than on what it can do in quiet accomplishment.

Insofar as we recognize our duties and capacities, so others will evaluate them. Of the past and present we can speak well. Men of unusual energy, vision, good will, and creative capacity have gone before and are among us. For the future I have no fear if, in our individual capacities, we have some care for the greater structure of which we form a part. We hold a trust which, if developed and nurtured with broad understanding, will serve a valuable and essential need and assure the continued success of this Association.

We are now assembled for our twenty-fifth annual meeting, and a perusal of the program indicates that those responsible for the numerous arrangements have worked diligently and efficiently to provide an instructive and entertaining week. It is my pleasure at this time to extend felicitations to our sister organization, the American Society of Brewing Chemists, which is meeting concurrently with us and with which a joint technical session has been arranged on Tuesday morning.

As the speaking voice of the American Association of Cereal Chemists, I desire to express to their members our keen interest in their work, to assure them of our cooperation, and to extend a hearty invitation to participate with us in all the scheduled events of the week.

In closing I cannot more aptly express the relation of our Association to the cereal industries than by quoting from an address by Mr. James Ford Bell, Chairman, Board of Directors, General Mills, Inc., before the Northwest Section, on March 31st last.

"Scientific research is industry's secret of perpetual youth. Without the constant infusion of new ideas from the laboratory and the testing plant, industry grows old, and progressively loses its vitality, until it is finally laid away as a useless relic of the past. It is industry's business to make and sell, but it is the business of science to teach industry what to make, and how to make its products better. It is to the scientists that the flour and cereal milling industry looks, and must look, for its chief guidance in the future if it is to hold and strengthen its position as one of the great servants of mankind."

# MINUTES OF THE TWENTY-FIFTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF CEREAL CHEMISTS

The President Hotel, Kansas City, Missouri

May 22 to 26, 1939

J. M. DOTY, *Secretary*

The largest group ever in attendance at a meeting of the American Association of Cereal Chemists gathered for the opening of the Silver Anniversary Meeting at the President Hotel in Kansas City, Missouri. President W. F. Geddes called the meeting to order at 9:40 a.m. on Monday, May 22, 1939. The invocation was given by the Rev. David H. Owen.

Dr. Geddes then asked all to stand for one moment of silence in remembrance of Dr. A. D. Barbour, who had died during the year.

The President introduced the Hon. Bryce B. Smith, Mayor of Kansas City, who welcomed all members and friends to the city.

The Secretary was called on to read letters and telegrams from the following: C. W. Partridge, Secretary of the Association of Operative Millers, who extended their best wishes; L. D. Whiting, a past president who could not attend this year's meeting but who wanted to wish us a most successful convention; and Swen Young, Chairman of the Toronto Section. Kent Jones also sent regrets that he could not meet with us.

Dr. Geddes then read his Presidential Message, printed in this issue of *Cereal Chemistry*.

Perie Rumold, Chairman of the Local Arrangements Committee, made announcements about the plans for the picnic and golf tournament to be held that afternoon.

Dr. Geddes introduced R. J. Clark, Past President, who in turn introduced all past presidents attending the meeting. They were C. J. Patterson, R. W. Mitchell, S. J. Lawellin, M. J. Blish, M. A. Gray, C. G. Harrel, R. K. Durham, R. C. Sherwood, Mary M. Brooke, Washington Platt, and C. H. Bailey. These people were called to the speaker's platform while Mr. Clark gave his address on the subject, "Achievements."

Next Dr. Geddes introduced Dr. E. R. Weidlein of the Mellon Institute, who talked on "Advance through Scientific Research." A copy of Dr. Weidlein's talk will be found in the Proceedings of the Twenty-Fifth Annual Meeting of the American Association of Cereal Chemists.

Dr. Geddes then introduced Dr. John H. Parker, who presented a paper entitled "The Cereal Chemist and Wheat Improvement."

Our guest speaker, William Hauck, President of the American Society of Bakery Engineers, was then introduced. He presented a paper entitled "Appreciation of the Contribution of Cereal Chemistry to the Baking Industry."

Dr. Geddes recessed the morning meeting at 12:30 p.m.

Monday afternoon was taken up with the picnic at the Hillcrest Country Club. Included in the afternoon's entertainment were the golf and pistol tournaments, bingo games, shooting exhibitions, awarding of prizes, etc.

## TUESDAY, MAY 23

The Tuesday morning session was a joint meeting with the American Society of Brewing Chemists. The meeting was called to order by Dr. Geddes, who welcomed the brewing chemists and expressed his appreciation of their excellent cooperation. He then introduced G. S. Bratton, President of the Society of Brewing Chemists, who thanked the American Association of Cereal Chemists for the opportunity of meeting with them in joint session.

Dr. Geddes introduced Phillip P. Gray, member of the Society of Brewing Chemists, who presided over this session.

Dr. Gray introduced the rest of the speakers on this program.

Paper—"Time Lapse Motion Photo-Micrographic Studies of Yeast Cells," by Charles N. Frey, George W. Kirby, William Schanzenbach, and Freeman Swift. Read by George W. Kirby.

Paper—"Barley and Malt Studies. VI. Experimental Malting of Barleys Grown in 1938," by J. G. Dickson, A. D. Dickson, H. L. Shands, and B. A. Burkhart. Read by A. D. Dickson.

Paper—"A Modification of the Wohlgemuth Method for the Determination of Alpha-Amylase and Comparison with a Viscosity Method," by Lawrence E. Ehrnst, George J. Yakish, William Olson. Read by Lawrence E. Ehrnst.

Paper—"A Standardized Wohlgemuth Procedure for Alpha-Amylase Activity," by R. M. Sandstedt, Eric Kneen, and M. J. Blish. Read by R. M. Sandstedt.

Paper—"Electrometric Determination of Diastatic Power of Malts," by B. A. Burkhart.

Paper—"Heat Inactivation of Enzymes During Mashing," by Kurt Becker, Ralph G. Swanson, and Thomas P. Kruzic. Read by Kurt Becker.

Paper—"Polariscopic Determination of Proteolytic Activity," by Quick Landis.

Paper—"Protein Modification of 1939 Mid-Western Malts," by Stephen Laufer.

Paper—"Laboratory Malting. III. Steeping Equipment and Method," by J. A. Anderson and W. O. S. Meredith. Read by W. O. S. Meredith.

Paper—"A Chemical Method for Determining Pasteurization Effects," by Robert I. Tenney, Nicholas S. Yanick, and Fred A. Wilcox. Read by Robert I. Tenney.

Paper—"The Role of Cereal Adjuncts in the Brewing Process," by Louis Ehrenfeld.

Paper—"Practical Research in the Brewery," by George B. Sippel.

Paper—"A Review of Modern Methods in the European Brewery Laboratory," by Bertel Krause. Dr. Krause accompanied his talk with very interesting pictures taken in some of the European laboratories.

On account of the length of the morning session, Dr. Krause's second paper was held over for the afternoon session. Dr. Gray turned the meeting over to Dr. Geddes, who recessed the meeting until 2:00 p.m. at 12:55.

After calling the afternoon meeting to order at 2:05 p.m. Dr. Geddes turned the proceedings over to C. H. Bailey, who presided at this session. Dr. Bailey read the paper which was to have been given by G. W. Scott Blair, who was unable to attend because of the sudden illness of his wife.

Paper—"Psychorheology in the Bread Making Industry," by G. W. Scott Blair. Read by C. H. Bailey.

Paper—"The Value of the Normal Farinogram for Predicting the Baking Strength of Western Canadian Wheats," by W. F. Geddes, T. R. Aitken, and M. H. Fisher. Read by W. F. Geddes.

Paper—"Quality Tests on Hard Winter Wheat," by R. K. Larmour, E. B. Working, and C. W. Ofelt. Read by R. K. Larmour.

Paper—"Further Observations on Dough Structure—a New Method of Standardization of Dough Properties," by C. W. Brabender.

Paper—"Plasticity of Doughs," by O. E. Stamberg and C. H. Bailey. Read by O. E. Stamberg. This paper finished the Symposium on Physical Methods Applied to Dough Properties and Wheat Quality Evaluation.

The paper scheduled for the morning session by Dr. Krause was introduced and presented at this time.

Paper—"Diastase in Barley and Wheat and Its Liberation," by Bertel Krause.

Paper—"The Proteinase Content of Wheat Flour," by W. S. Hale.

Paper—"A Review of the 1938 Literature Pertaining to the Field of Cereal Chemistry," by W. S. Hale.

Dr. Bailey recessed the Tuesday session at 5:25 p.m.

### WEDNESDAY, MAY 24

President Geddes called the meeting to order at 9:15 a.m. He then called on the Secretary to read the minutes of the last meeting. The Secretary moved that the reading of the minutes be waived and that the minutes as printed in the July, 1938, issue of *Cereal Chemistry* be approved without reading. Seconded by G. F. Garnatz. Carried.

The Chairman called for a report from the Treasurer. Dr. Skovholt moved that the treasurer's report as printed in the March, 1939, issue of *Cereal Chemistry* be accepted. Seconded by R. J. Clark. Carried. Dr. Skovholt again pointed out the need for action in preparing to revise and reprint "Cereal Laboratory Methods."

The Auditing Committee was called on to make a report. That report was read by the Secretary, who moved its adoption. Seconded by C. A. Glabau. Carried.

President Geddes called for a report from the Editor-in-Chief of *Cereal Chemistry*. M. J. Blish moved the adoption of his report. Seconded by Avery Dunn. Carried.

The report of the Managing Editor was read by R. M. Sandstedt, who moved the adoption of his report. Seconded by R. H. Harris. Carried.

D. A. MacTavish made a report on the work of the Membership Committee and moved the adoption of this report. Seconded by Paul Logue. Carried.

C. A. Glabau was called on to report the work of the Committee on Employment. Mr. Glabau moved this report be adopted. Seconded by Lawrence Zeleny. Carried.

R. W. Mitchell read a report from the Committee on the Osborne Medal Award. Mr. Mitchell moved the adoption of this report. Seconded by J. T. Flohil. Carried.

A report from the Inter-Relations Committee was called for but since no member of this committee was present, the report was postponed.

R. J. Clark read and moved the adoption of the report of the History Committee. Seconded by W. Platt. Carried.

President Geddes called on Mary Brooke, who read the report of the Committee on Cooperative Research and moved its adoption. Seconded by G. E. Findley. Carried.

Paul Logue, Chairman, made a report for the Investment Committee and moved its adoption. Seconded by H. D. Liggitt, Jr. Carried.

Victor E. Marx read and moved the adoption of the report of the Publicity Committee. Seconded by J. A. Dunn. Carried.

In the absence of G. N. Bruce, Secretary Doty read the report of the Traffic Committee and moved adoption of the report. Seconded by Paul Logue. Carried.

Dr. Geddes called on R. C. Sherwood, Chairman, to report on the work of the Committee on Requirements for Membership. Dr. Sherwood read the recommendations and moved their adoption. He explained that the adoption of this report in no way bound the members to make the constitutional changes recommended but merely approved the work of the committee. Seconded by J. A. Shellenberger. Carried.

The Vice-President, G. F. Garnatz, Chairman of the Executive Committee, made and moved the adoption of the report of the work of that committee. Seconded by C. N. Frey. Carried.

C. G. Harrel reported for the Inter-Relations Committee and moved adoption of his report. Seconded by W. L. Heald. Carried.

Dr. Bailey moved that the amendments to the Constitution be made section by section rather than as a whole. Seconded by H. D. Liggitt. Carried.

Dr. Bailey moved that Article III, Section 1, be changed to read, "The Membership of this Association shall be divided into three classes, (1) active, (2) honorary, and (3) sustaining." Seconded by R. W. Mitchell. Carried.

Dr. Bailey moved that Article III, Section 2, be changed to read, "Eligibility for active membership in this Association (subsequent to May 24, 1939) shall be restricted to

"(a) those persons having the Bachelor's degree with a major in science from a college, university, or technical school of recognized standing, or having at least four years of collegiate training with a major in science in such an educational institution.

"(b) those persons presenting evidence that they have had at least two years of training in chemistry, plus four years of practical experience in a chemical laboratory involving varied chemical and technological determinations." Seconded by H. K. Parker after considerable discussion. Carried.

Dr. Bailey moved that Article III, Section 3, be changed to read, "Application for active membership shall be made in writing, endorsed by at least one active member in good standing, accompanied by the proper fee and directed to the Treasurer, who in turn shall refer the application to the Membership Application Committee, its decision to be final, and upon report to the Treasurer to be transmitted by him to the candidate." Dr. Bailey then pointed out that at the time the above change was voted on the members should also vote on Article IV, Section 8, which should be changed to read, "Membership Application Committee. The President shall appoint a Membership Application Committee, consisting of three active members, two of whom shall be past national officers, the duties of said Committee to be the examination of applications for membership, with full authority to approve or disapprove, in accordance with the provisions of Article III, all applications submitted to it by the

Treasurer. Upon reaching a decision the Committee shall notify the Treasurer, who shall inform the candidate of the disposition of his application." Both motions were seconded by C. N. Frey. Carried.

Dr. Bailey moved that Article III, Section 4, be changed to read, "Honorary members may be elected by a three-fourths majority vote of the active members present at any general meeting, the candidate to be first proposed by one or more active members to the Executive Committee, who in turn shall report on the qualifications of the candidate to the general meeting." Seconded by W. Platt. Carried.

Dr. Bailey moved that Article III, Section 5, be changed to read, "Corporations, institutions or individuals who are interested in or concerned with the use of cereals or cereal products may become sustaining members upon application to the Treasurer, in accordance with Section 3, Article III." Seconded by W. M. Cathcart. Carried.

Dr. Bailey moved that Article V, Section 4, be changed to read, "Sustaining members. The dues of sustaining members shall be ten dollars per annum, from January first to December thirty-first. Said dues are payable in advance, and shall be allotted to the publication fund." Seconded by F. C. Hildebrand. Carried.

Dr. Bailey moved that Article VI, Section 5, be changed to read, "Privileges of Honorary and Sustaining Members.

"(a) Honorary members, sustaining members (and/or their representatives) shall have the privilege of attending all general meetings and in addition the privilege of the floor, but shall have no vote.

"(b) Honorary and sustaining members shall be entitled to receive the regular publications of the Association and may have the privileges of membership in a local section." Seconded by P. Logue. Carried.

Mr. Garnatz moved that Article IV, Section 1, be changed to read, "The elective officers of this Association shall be six in number: namely, President, Vice-President, Secretary, Treasurer, Editor-in-Chief, and Managing Editor of *Cereal Chemistry*. All officers except the Treasurer shall assume the duties of their offices at the end of the Annual Convention. The Treasurer's term shall be for the calendar year following his election." Seconded by J. A. Dunn. Carried.

Mr. Garnatz moved that Article IV, Section 4 (d), be changed to read, "The Treasurer shall collect all fees and moneys due the Association, and pay all bills by check, and shall record all such receipts and expenditures. Bills shall not be paid except with the approval of the Vice-President. The Treasurer shall also keep the membership roster of the Association. Routine duties involving the collection of dues and keeping of membership records may be delegated by the Treasurer to an Assistant, at the discretion of the Executive Committee." Seconded by W. Platt. Carried.

Quick Landis moved to delete the word *Treasurer* after the word *Assistant* in the above change. Seconded by M. J. Blish. Carried. Therefore the above change stands.

Mr. Garnatz moved that Article IV, Section 5, be changed to read, "(d) The Executive Committee may, at their discretion, employ an Assistant for the transaction of such detailed business and financial affairs of the Association as may be delegated to him by the Executive Committee." This proposed change was included in the motion made above by Mr. Garnatz regarding the change in Article IV, Section 4 (d). Both amendments were seconded by W. Platt, and carried.

Dr. Geddes then called for the report of the Nominating Committee. R. W. Mitchell made the following nominations and moved their adoption as the report of his committee: President, G. F. Garnatz; Vice-President, C. F. Davis and Paul Logue; Secretary, J. M. Doty; Treasurer, Oscar Skovholt; Editor-in-Chief, M. J. Blish; Managing Editor, R. M. Sandstedt. Seconded by H. K. Parker. Carried.

Pearl Brown moved the nominations for President be closed and that the Secretary be instructed to cast a unanimous ballot for George Garnatz for President. Seconded by C. G. Ferrari. Carried.

J. R. Davies nominated C. H. MacIntosh for Vice-President. Seconded by J. A. Dunn. Carried. The names appearing on the first ballot for Vice-President were Paul Logue, C. F. Davis, and C. H. MacIntosh. Acting tellers were C. G. Ferrari, M. A. Gray, and C. H. Bailey. The first ballot gave MacIntosh 55 votes, Davis 54, and Logue 50.

Dr. Frey moved that all names be left on the ballot for the second time. If no man was elected on the second ballot, then the low man should be removed for the third ballot. Seconded by C. O. Stamberg. Carried. The second ballot gave

MacIntosh 57 votes, Davis 54, and Logue 51. Paul Logue's name was removed from the third ballot.

During the time of the third balloting, President Geddes called on C. F. Davis, Chairman of the Committee on Standardization of Laboratory Baking, to give his general report of the work of his committee. The three papers following Mr. Davis' report were also given during the balloting.

Paper—"Control of Dough Temperatures in Test Baking," by J. G. Malloch.

Paper—"The Use of Hand Operated Sheetting Rolls in Test Baking," by J. A. Shellenberger.

Paper—"Studies of the Usefulness of a Motor-Driven Sheeter in Test Baking," by Paul P. Merritt and Max C. Markley. Read by Max C. Markley.

The Chairman called on the tellers to report the result of the third ballot, and this report showed C. F. Davis the new Vice-President. Nominations for Secretary were opened. H. D. Liggitt moved the nominations be closed and that the Secretary be instructed to cast a unanimous ballot for J. M. Doty as Secretary. Seconded by W. Platt. Carried.

Dr. Geddes opened the nominations for Treasurer. E. B. Working moved the nominations for Treasurer be closed and that the Secretary be instructed to cast a unanimous ballot for Oscar Skovholt for Treasurer. Seconded by E. E. Smith. Carried. The nominations for Editor-in-Chief of *Cereal Chemistry* were opened. Paul Logue made the motion that the Editor-in-Chief and Managing Editor be elected at the same time since they worked together so closely. He moved that the nominations for these offices be closed and that the Secretary cast a unanimous ballot for M. J. Blish and R. M. Sandstedt for these offices in the order named. Seconded by C. H. MacIntosh. Carried.

R. C. Sherwood moved that Oscar Skovholt be appointed Treasurer until the end of the calendar year by the Executive Committee. Seconded by C. N. Frey. Carried.

W. Platt moved that the business session be closed. Seconded by M. A. Gray. Carried.

Dr. Geddes called C. F. Davis to the platform for a few words. Mr. Davis thanked the members and promised his best efforts for the Association. Dr. Geddes then called Dr. Larmour to the speaker's table to take over his duties as Presiding Chairman of the morning session. Dr. Larmour called on Dr. Markley for the next paper.

Paper—"Report on a Compromise Test Baking Pan," by M. C. Markley.

Paper—"Gas Retention as Affected by Including Shortening in the Tentative A.A.C.C. Basic Test Bake Formula," by W. L. Heald. This concluded the papers given by the Committee on Standardization of Laboratory Baking.

Paper—"A Study of Baking Methods for Evaluating the Quality of Hard Winter Wheats," by K. F. Finney and Mark A. Barmore. Read by Mr. Finney.

Dr. Larmour turned the meeting back to President Geddes, who recessed the meeting until 2:15 p.m. Recess was called at 1:10 p.m.

The afternoon session was opened at 2:15 p.m. with President-elect Garnatz presiding. He called on H. W. Putnam, Chairman of the Committee on Methods of Testing Soft Wheat, for his general report. Mr. Putnam, however, said he would give his report after the papers given by the members of his committee. Mr. Garnatz then called for the following papers:

Paper—"Report of Sub-Committee on Methods of Testing Cake Flours," by J. W. Montzheimer. Read by W. E. Stokes.

Paper—"Standardization of Methods for the Scoring of Test Cakes," by O. E. Stenberg.

Paper—"Comparative Study of the Results Obtained with Seven Commercial Type Cake Formulas in the Evaluation of Cake Flour Quality," by Francis J. Coughlin and Donald Wade. Read by Donald Wade.

Paper—"Discussion of Display of Cakes Baked from Seven Commercial Cake Formulas," by William R. Green.

Paper—"Report of Sub-Committee on Methods of Testing Self-Rising and Phosphated Flours," by O. E. Gookins.

Paper—"Report of Sub-Committee on Methods of Testing Biscuit and Cracker Flours," by Howard M. Simmons.

Paper—"General Report of the Committee on Methods of Testing Soft Wheat Flours," by H. W. Putnam. This summary concluded the papers presented by this committee. Mr. Garnatz introduced the next paper on the program.



Paper—"Checking," presented by Jan Micka.

Paper—"Fat Acidity in Relation to Heating of Corn in Storage," by Lawrence Zeleny. Because of the length of the afternoon program, Mr. Garnatz asked Dr. Bayfield if he could present his paper the first thing on the Thursday morning session. Dr. Bayfield agreed and Mr. Garnatz recessed the Wednesday session at 5:25 p.m.

At 7:00 Wednesday evening, the annual dinner dance was held in the Roof Garden of Hotel President. The Local Arrangements Committee had planned an excellent program including dinner, floor show, and dancing.

#### THURSDAY, MAY 25

Dr. Sherwood, Chairman, called the meeting to order at 9:17 a.m. He immediately called on Dr. Bayfield for his paper, which had been held over from the Wednesday afternoon session.

Paper—"Observations Indicating the Need for Controlled Mill Room Conditions in Experimental Milling," by E. G. Bayfield.

The next part of the program was turned over to the Committee on Methods of Analysis, R. M. Sandstedt, General Chairman. Dr. Sherwood called for the following papers:

Paper—"Collaborative Study of the 15-Minute Moisture Method in Comparison with the Official Air-Oven Method II," by H. W. Putnam.

Paper—"A Physical Method of Determining Flour Value, Making Use of Gas Retention," by W. L. Heald.

Paper—"Proteolytic Activity Determinations in Cereal Products," by F. C. Hildebrand.

Paper—"The Pressure Meter in the Study of Yeast," by J. M. Doty and W. R. Urban. Read by J. M. Doty.

Paper—"General Report of the Committee on Methods of Analysis," by R. M. Sandstedt. Dr. Sherwood then called on Elsie Singruen to make a report for the Malt Analysis Standardization Committee. Mr. Rumold, Chairman of the Local Arrangements Committee, made some announcements and asked the members to decide on their preferences as to the inspection trips scheduled for the afternoon. Dr. Sherwood called for the following papers:

Paper—"Vitamin B<sub>1</sub> Content of Wheat, Flour, and Bread," by Alfred Shultz, Lawrence Atkin, and C. N. Frey. Presented by Dr. Frey.

Paper—"A Method of Determining Vitamin B<sub>1</sub> using the Fluorophotometer," by D. J. Hennesy.

Paper—"Some Applications of Time Lapse Ciné-Photography to Cereal Chemistry," by William Schanzenbach and Quick Landis. Read by Dr. Landis.

Paper—"Flour Changes on Storage in Different Types of Bags," by William H. Cathcart. Dr. Sherwood recessed the morning session at 11:55 p.m.

The Presidents' Luncheon was held in the Walnut Room of the hotel for the installation of the new officers. During the luncheon R. A. Barackman made and moved adoption of the following resolution:

"Inasmuch as the American Association of Cereal Chemists is deeply indebted to Dr. W. F. Geddes for the excellent manner in which he has directed the affairs of the Association during the past year,

"Be it resolved that we, the members of the Association, hereby express to Dr. Geddes our sincere appreciation of the many services which he has rendered in our behalf;

"Be it further resolved that a memorial be prepared and presented to Dr. Geddes whereby his services to the Association will be suitably commemorated."

Thursday afternoon was taken up with inspection trips to the Sheffield Steel Company, Corn Products Refining Company, the Research Laboratory of Campbell-Taggart Research Corporation, and the Goetz Brewing Company, all of Kansas City.

#### FRIDAY, MAY 26

Officers of the National Association met with the officers of the Local Sections for breakfast in Room 229, Hotel President, at 8 a.m.

Dr. Blish, presiding officer for the Friday morning session, called the meeting to order at 9:20 a.m. He immediately called for the following papers.

Paper—"Dough Oxidation and Mixing Studies. IV. Effect of Oxygen and Oxidizing Salts on Sponge Doughs," by J. Freilich and C. N. Frey. Read by J. Freilich.

Paper—"Effect of Temperature on Dough Properties, II," by M. D. Mize and J. C. Baker. Given by J. C. Baker.

Paper—"Maltose Fermentation in the Dough," by A. L. Shultz, Lawrence Atkin, and C. N. Frey. Read by C. N. Frey.

Paper—"Effect of Fumigation on Whole Wheat Flour," by Frank James.

Paper—"The Permeability of Bread by Air," by J. C. Baker.

Paper—"Effect of Storage Temperatures upon the Viability and Baking Properties of Compressed Yeast," by M. T. Bartram, S. C. Rowe, and L. H. Bailey. Read by L. H. Bailey.

Dr. Blish turned the meeting back to Dr. Geddes. Because some of the members had expressed the desire to get away as soon as possible after the afternoon session, Dr. Geddes suggested that that session start earlier than scheduled. Members indicated by a hand vote that they would like to start at 1:15 p.m. instead of at 2:00 p.m. The morning meeting was recessed at 11:45.

The Friday afternoon session was called to order at 1:30 by W. L. Heald, the presiding officer. He called on Washington Platt, Chairman of the Resolutions Committee, to make a report for that committee. Mr. Heald then called on the speakers who presented the following papers.

Paper—"Starch in Relation to Some Baking Properties of Flour," by R. M. Sandstedt, C. E. Jolitz, and M. J. Blish. Read by R. M. Sandstedt.

Paper—"Starch as a Factor in Dough Formation," by O. E. Stamberg.

Paper—"Studies on Wheat Starch Fractions," by O. E. Stamberg. Mr. Heald then turned the meeting over to Dr. Geddes for the business session. Dr. Geddes asked for formal adoption of the report of the Committee on Methods of Analysis. J. M. Doty moved the adoption of the report as given by Mr. Sandstedt. Seconded by C. H. MacIntosh. Carried. W. O. S. Meredith moved the adoption of the report of the Malt Analysis Standardization Committee as given by Miss Singruen. Seconded by Quick Landis. Carried. Quick Landis gave a report for the Committee on Definition of Technical Terms. Dr. Landis moved the adoption of this report. Seconded by M. A. Gray, carried. C. F. Davis, Chairman of the Committee on Standardization of Laboratory Baking, moved the adoption of the report as given. Seconded by Elise Shover. Carried. Mr. Putnam moved the adoption of the report of the Committee on Methods of Testing Soft Wheat Flour and its three Sub-Committees. Seconded by Oscar Skovholt. Carried. Washington Platt moved the adoption of the Report of the Resolutions Committee. Seconded by C. F. Davis. Carried.

Dr. Geddes called C. H. MacIntosh, Chairman of the Program Committee, to the platform and thanked him on behalf of the Association for the excellent program he had arranged. Dr. Geddes also thanked everyone who had served on Mr. MacIntosh's committee. Mr. MacIntosh thanked the Association members for placing their confidence in him and said that he had enjoyed the work. Dr. Geddes then asked those present to applaud Mr. MacIntosh to show their appreciation of his efforts. Dr. Geddes asked for Perie Rumold, but since he was not present, Dr. Geddes expressed the appreciation of the Association to W. L. Heald of the Local Arrangements Committee. The retiring president thanked Mrs. Rumold for the excellent program she and her committee had arranged for the women present at the Annual Meeting.

Dr. Geddes thanked the members for their fine cooperation and good fellowship during his term as President. He then turned the gavel over to the new president, Mr. Garnatz.

President Garnatz gave a partial list of the various committee appointments. He pointed out that two of the committees would be discontinued, namely the Committee on Requirements for Membership and the Traffic Committee.

J. A. Dunn moved that the meeting adjourn. Seconded by D. A. MacTavish. Carried. President Garnatz adjourned the Twenty-Fifth Annual Meeting at 3:35 p.m.

## REGISTRATION OF CONVENTION, KANSAS CITY, MO.

May 22-26, 1939

## MEMBERS

R. C. Alban	J. A. Dunn	H. W. Kingsbury
F. W. Albro	R. K. Durham	G. W. Kirby
Geo. L. Alexander		R. N. Knudson
J. A. Anderson	W. G. Epstein	A. L. Lancaster
Arlee A. Andre	C. F. Evert	Q. Landis
Hy H. Arendall		R. K. Larmour
C. A. Armstrong	H. S. Faulkner	Stephen Laufer
Lowell Armstrong	C. G. Ferrari	S. J. Lawellin
Charles C. Armuth	G. E. Findley	F. H. Lawford
W. G. Artis	K. F. Finney	L. E. Leatherock
	V. E. Fisher	H. L. Lentz
C. H. Bailey	Henry Flick	H. D. Liggitt
L. H. Bailey	J. T. Flohil	Paul Logue
John C. Baker	E. N. Frank	K. H. Lorenz
R. A. Barackman	J. Freilich	H. J. Loving
M. A. Barmore	C. N. Frey	J. M. Lugenbeel
E. G. Bayfield	R. L. Frye	F. J. Lumsden
Kurt Becker		
H. N. Bergsteinsson	Miss B. Galletly	C. H. MacIntosh
J. P. Bishop	Geo. Garnatz	D. A. MacTavish
M. J. Blish	W. F. Geddes	M. C. Markley
R. T. Bohn	H. O. Gilmer	Miss E. L. Martin
O. R. Borngesser	C. A. Glabau	Victor E. Marx
L. R. Bowman	E. F. Glabe	J. E. Mastin
D. L. Boyer	Phil Goodwin	R. E. McCormick
C. W. Brabender	O. E. Gookins	Harold W. McGhee
T. A. Brantley	M. A. Gray	R. M. McKinstrie
G. S. Bratton	P. P. Gray	L. H. McLaren
C. L. Brooke	W. R. Green	Nell McNeil
Mary M. Brooke	N. L. Gregory	W. O. S. Meredith
E. B. Brown	Arthur W. Gust	J. B. Merryfield
Lionel G. Brown		W. C. Meyer
Pearl Brown	L. W. Haas	Jan Micka
P. J. Buchanan	T. L. Haberkorn	P. E. Minton
Geo. H. Buford	Harold Hall	R. W. Mitchell
Howard Burrus	Robt. W. Haman	M. D. Mize
F. C. Buzzelle	C. G. Harrel	Elmer Modeer
	R. H. Harris	G. Moen
Frank Carr	William Hauck	Claude L. Moore
W. H. Cathcart	W. L. Heald	Ray E. Moser
Howard A. Clark	F. C. Hildebrand	
Lee E. Clark	E. V. Holm	C. T. Newell
Rowland Clark	Ray Horn	Harry Obermeyer
W. S. Claus	Geo. L. Howard	C. O. Oppen
F. A. Collatz	H. P. Howells	T. J. Otterbacher
Winthrop R. Corey	H. S. Hutchinson	T. N. Ovlen
C. H. Crawford		
A. E. Curtis	Bert D. Ingels	H. K. Parker
	Roy Irvin	M. H. Parlin
John R. Davies		C. W. Partridge
Claud F. Davis	L. E. Jackson	C. J. Patterson
G. A. Davis	Frank James	F. D. Patterson
W. J. Davis	W. G. Jantzen	Frank Paul
A. D. Dickson	H. H. Johnson	Earl C. Paulsel
J. M. Doty		Grant W. Pearcy
F. L. Dunlap	Chas. H. Keipper	
J. O. Dunlap	A. J. King	

L. W. Pingree  
 Perie R. Pitts  
 Washington Platt  
 R. B. Potts  
 R. A. Pouchain  
 Ray Powers  
 H. W. Putnam  
 Glenn L. Pyle

W. L. Rainey  
 O. H. Raschke  
 Christian Rask  
 L. Reimers  
 Whitman Rice  
 C. E. Rich  
 Thos. C. Roberts  
 W. L. Roberts  
 Hugo W. Rohde  
 W. C. Rohrbaugh  
 K. S. Rohrbough  
 Perie Rumold

R. M. Sandstedt  
 T. W. Sanford  
 A. R. Sasse  
 J. S. Schlesinger  
 J. A. Shellenberger

R. C. Sherwood  
 V. Shiple  
 Elise Shover  
 Fred P. Siebel, Jr.  
 R. V. Siebel  
 Howard M. Simmons  
 Miss E. Singruen  
 O. P. Skaer  
 Oscar Skovholt  
 E. E. Smith  
 S. R. Snider  
 Samuel K. Sosland  
 G. R. Stadler  
 O. E. Stamberg  
 W. R. Steller  
 W. E. Stokes  
 Joe Stoklas  
 J. D. Stone  
 D. F. Stutzman  
 Betty Sullivan  
 C. O. Swanson

G. D. Thevenot  
 W. Tholstrup  
 L. M. Thomas  
 E. F. Tibbling  
 W. M. Tinkham

A. A. Towner  
 Carl E. Turner  
 L. C. Tuttle

G. G. Van Patten  
 W. V. Van Scoyk  
 C. G. Vaupel  
 E. A. Vaupel

Donald Wade  
 C. C. Walker  
 H. G. Walter  
 Jack Ward  
 T. R. Warren  
 Peter J. F. Weber  
 John S. Whinery  
 J. W. Whitacre  
 Robert Whiteside  
 H. M. Wight  
 Martin Wise  
 Carl E. Witter  
 E. B. Working  
 W. W. Worzella  
 Charles B. Wright

Lawrence Zeleny

#### NON-MEMBERS

E. G. Belt  
 Mrs. G. S. Bratton  
 Mrs. C. L. Brooke  
 Mrs. E. B. Brown  
 Mrs. G. H. Buford  
 G. M. Burkert  
 B. A. Burkhart  
 Mrs. Howard Burrus

R. D. Callaway  
 G. T. Carlin  
 Mrs. Rowland Clark  
 Mrs. W. S. Claus  
 Mrs. F. A. Collatz  
 Louis Comaschi  
 Mrs. A. E. Curtis

Mrs. Claud Davis  
 Mrs. J. M. Doty  
 V. F. Duensing  
 Mrs. R. K. Durham

Joseph Eichberg  
 J. J. Enright  
 Mrs. C. F. Evert

F. H. Faber  
 Floyd Fassnacht  
 Mrs. Floyd Fassnacht  
 J. B. Ferguson  
 Tom Ferguson  
 Mrs. C. G. Ferrari  
 Mrs. G. E. Findley

Mrs. J. T. Flohil  
 Mrs. E. N. Frank  
 Mrs. Rolfe L. Frye

Mrs. Geo. Garnatz  
 Molly Geddes  
 Mrs. W. F. Geddes  
 Mrs. O. E. Gookins  
 Bob Graham  
 Mrs. P. P. Gray  
 Mrs. Wm. R. Green  
 Mrs. Arthur Gust

Mrs. L. W. Haas  
 Walter S. Hale  
 Mrs. C. G. Harrel  
 Mrs. W. L. Heald  
 Mrs. E. V. Holm  
 Mrs. Ray Horn  
 Mrs. Geo. L. Howard  
 Mrs. H. P. Howells

Harry E. Inman

Mrs. L. E. Jackson  
 Mrs. W. G. Jantzen  
 Charles E. Jolitz  
 L. M. Josephson  
 F. W. Jung

J. C. Kintz  
 Mrs. J. C. Kintz

Mrs. Q. Landis  
 Mrs. L. E. Leatherock  
 Mrs. H. D. Liggitt  
 Mrs. Paul Logue  
 Mrs. H. J. Loving

Mrs. C. H. MacIntosh  
 H. L. Marks  
 Mrs. H. L. Marks  
 Mrs. Victor Marv  
 D. J. Maveety  
 Harris McGavock  
 Mrs. Harris McGavock  
 Mrs. Harold W. McGhee  
 Mrs. R. M. McKinstrie  
 Mrs. L. H. McLaren  
 Mrs. J. B. Merryfield  
 Mrs. W. C. Meyer  
 G. E. Mickle  
 Mrs. R. W. Mitchell  
 Mrs. G. Moen  
 Miss Edna Montgomery  
 Mrs. Ray E. Moser  
 V. E. Munsey

Milton E. Nelson  
 Mrs. Milton E. Nelson  
 Mrs. C. T. Newell  
 A. I. Newman  
 H. E. Noelck

Mrs. Harry Obermeyer  
 Frank Osborn

John H. Parker  
Geo. T. Peckham  
A. Pitann  
Mrs. R. B. Potts  
Mrs. R. A. Pouchain  
R. W. Pratt  
Mrs. R. W. Pratt  
Mrs. Glenn L. Pyle

Mrs. O. H. Raschke  
Mrs. Christian Rask  
Mrs. Whitman Rice  
Mrs. W. L. Roberts  
G. C. Robinson  
Mrs. K. S. Rohrbough  
Mrs. Perie Rumold

M. K. Sanders  
Mrs. T. W. Sanford  
Mrs. A. R. Sasse  
Mrs. J. S. Schlesinger  
Roland Selman  
Mrs. R. C. Sherwood  
Bill Small  
C. C. Smelzer  
Mrs. Edward Smith  
Mrs. Geo. Stadler  
Mrs. O. E. Stamborg  
Mrs. W. R. Steller  
Mrs. W. E. Stokes  
Mrs. D. F. Stutzman

Robert I. Tenney

W. L. Thompson  
Mrs. E. F. Tibbling  
Mrs. A. A. Towner  
Mrs. Carl Turner  
Mrs. L. C. Tuttle

Mrs. G. G. Van Patten  
Mrs. C. G. Vaupel  
S. S. Voris  
Mrs. S. S. Voris

C. Weaver  
R. L. Watkins  
Mrs. John S. Whinery  
Mrs. J. W. Whitacre  
Mrs. Robert Whiteside

## REPORTS OF COMMITTEES

### The Executive Committee

GEO. GARNATZ, *Chairman*

President Geddes, in his address Monday morning, reported on the state of the Association and indicated that a detailed account of the business activities would be included in the report of the Executive Committee. The following therefore is submitted for the approval of the membership and constitutes a report of the actions taken by the Executive Committee in conducting interim business and passing on matters of policy.

Several proposals were submitted to the committee by mail and in addition meetings were held in Cincinnati on May 26, 1938; in Chicago on March 15, 1939; and in Kansas City on May 21, 1939.

With the knowledge and consent of the committee and on authorization by President Geddes, the Committee on Membership Requirements prepared the results of their studies on this subject in the form of constitutional revisions. Those portions of the constitution thus affected were set up in comparison with the revisions, and, in conformity with the requirements for amending the constitution, were mailed out to the membership in time to qualify for action at this meeting.

In last year's Executive Committee report, a recommendation was made that some consideration be given toward providing a part time paid employee to relieve some of the officers of certain burdensome routine duties and to eliminate duplication of effort through centralization of certain business activities. In accordance with this recommendation a constitutional amendment is proposed which will make it possible for the Executive Committee to appoint at their discretion an Assistant Treasurer.

These provisions are considered adequate and flexible and contemplate consolidation of this work in the office of the Managing Editor of *Cereal Chemistry*, elimination of duplication of effort there and in the treasurer's office and at the same time relieving the treasurer and secretary of burdensome routine details. It is the recommendation of the Executive Committee that favorable action be taken on this proposal.

The treasurer has recommended to the Executive Committee the desirability of having the treasurer assume office on a calendar-year basis rather than immediately following the annual meeting, in order to eliminate the mid-term audits and to permit a more orderly and business-like transfer of duties. It is recommended that favorable action be taken by the Association.

Twelve applications for membership which were referred to the Executive Committee were favorably received.

By invitation from the American Society of Bakery Engineers, the Association participated in their program largely through the Committee on Cooperative Research and also arranged for a paper on "Boiler Water Treatment, Scale Formation,

and Pipe Corrosion" by D. W. Haering. S. J. Lawellen also presented a paper dealing with the sanitation aspects of water supplies.

The budget for 1939 was adopted as presented by the treasurer.

The surplus (\$339.63) resulting from the Cincinnati meeting was turned over to the Executive Committee by the Cincinnati Section. This was accepted with thanks and allocated to the general fund of *Cereal Chemistry* to be used in printing additional papers at the discretion of the Editor-in-Chief.

It was agreed to close the account with the Harris Trust and Savings Bank of Chicago and, upon the recommendation of the Investment Committee, to invest \$4,500.00 of the Association's surplus funds in U. S. Savings Bonds yielding 2.9%.

Consideration has been given to a request for the separate publication of the reference tables now included as an appendix to *Cereal Laboratory Methods*. These tables can be provided in a separate binding at approximately seventy-five cents per copy, and it was decided to delay action until a canvass of possible sales was made.

The proposal that national officers visit local sections was discussed and the Executive Committee proposes to designate up to \$150.00 annually for this purpose, which is to meet approximately 50% of the expenses incurred, the participating sections to meet the rest of the expenses. Requests are to originate from the sections and will be passed on by the president of the Association.

It is realized that in certain sections the constitutional provision relative to the proportion of national association members required, is not being lived up to and accordingly the Executive Committee recommends to the succeeding committee that proper steps be taken to amend the constitution along such lines as are more practical and at the same time will assist the Membership Committee by encouraging the sections to seek eligible candidates who will ultimately seek membership in the national association.

Many routine matters of business were discussed and acted on, including arrangements for the present convention. Representatives of the New York Section renewed their invitation to the A. A. C. C. to meet in New York in 1940; invitations were also received to hold the 1940 meeting in Milwaukee, Louisville, Philadelphia, and Biloxi, Miss. In view of the overwhelming majority who favored New York in the post-card ballot, this city has been selected as our meeting place for 1940.

An invitation to meet in Omaha was received and filed for consideration when the rotation plan calls for the A. A. C. C. to meet in the southwest again and another invitation from Louisville as well as one from Columbus, Ohio, were similarly recorded.

The Auditing Committee recommended that in addition to the summarized items covering *Cereal Chemistry* in the treasurer's report, a detailed financial report covering the journal should be published. The editors of *Cereal Chemistry* and the Executive Committee concur in this recommendation.

Because of the necessity for the managing editor of *Cereal Chemistry* to be in close association with the editor-in-chief and because such an arrangement lends itself better to emergencies and transfer of duties the present Executive Committee recommends to the succeeding one that steps be taken to amend the constitution to make the office of Managing Editor appointive by the Executive Committee rather than elective.

In view of the increasing size of *Cereal Chemistry* and the increased membership, with resultant increased work for the Editor-in-Chief, Assistant Editor, and Managing Editor, the Committee recommends a more equitable remuneration for the services of these officers.

A communication was received from the group of Association members in Winnipeg, Canada, expressing the intent to form a Cereal Chemists' Club with the ultimate purpose in mind to form a Section when the group is firmly established.

Reports received by the secretary indicate that the several local sections are functioning actively and are cooperating closely with the Association officers and the Publicity Committee.

All in all it would appear that the affairs of the Association are being administered actively and that a healthy condition exists.

As chairman of the Executive Committee I desire to express my appreciation for the fine cooperation extended me by the members of the Committee and by Dr. Skovholt and Mr. Doty.

### The Committee on Investment

PAUL LOGUE, *Chairman*

Your committee recommended to the treasurer in February that the surplus funds then available be invested in bonds of the United States Government.

As further guidance in the investment policy of the Association in the future your committee suggests the following:

(1) Maintain a minimum average cash balance in the checking account equivalent to one month's average expenditures.

(2) Maintain in savings accounts the equivalent of three average months' expenditures.

(3) Maintain in readily marketable bonds of the United States Government funds equivalent to an average year's expenditures.

(4) After the above requirements have been met, invest funds to the extent of two years' expenditures in bonds of states, municipalities, and public utilities which have not defaulted on the interest of any bonds in the past 25 years, and which are approved for investment for insurance companies in the State of New York.

### The Auditing Committee

H. K. PARKER, *Chairman*

The Auditing Committee met with the treasurer February 16th for the 1939 audit. Our report in respect to the same will be found on page 300 of the March, 1939, issue of *Cereal Chemistry*.

In addition to this formal printed report we wrote to President Geddes that in our opinion there were funds available for investment and that such a move should be carried out to establish a safe limit, since the amount carried was well over the Federal Reserve guarantee of \$5,000.

We also suggested that a report such as prepared by the managing editor of *Cereal Chemistry* should be included in the general treasurer's report so that the picture of the business done in respect to our publication would be a little more clear to the membership.

### The Employment Committee

CHARLES A. GLABAU, *Chairman*

The Employment Committee has had 28 applications for positions from cereal chemists, from January, 1938, to May, 1939.

Twenty cereal chemists have applied for positions from June, 1938, to May, 1939. During this same period, eight companies requiring the services of cereal chemists have applied to the Employment Committee for applicants. The committee has not been able to determine how many of these positions have been filled by candidates suggested.

The employment situation appears to have been somewhat better for cereal chemists over the past year.

The committee has given some thought to the development of an application blank which may be used in determining the qualifications a candidate may have when applying for a position as a cereal chemist. The committee hopes to have such a blank completed very shortly.

### The Membership Committee

D. A. MACTAVISH, *Chairman*

Your committee's efforts this past year have been directed along the same channels as in 1937-38, namely, a continuous endeavor to bring home to the membership as a whole, but more particularly the officers of the local sections, the necessity of maintaining and adding to the roll of active members of our national association. To this end we have made use of the space allotted to our committee in the various issues of the News Letter, have circularized the sectional chairmen, and sent personal letters to prospectives whenever a name or list of names has been given to us.

The treasurer has turned over to our committee for further attention (both in 1938 and 1939) lists of delinquents who had not responded to "final notices" and these

ex-members have had personal appeals sent out to them or their delinquency has been called to the attention of the chairmen of the local sections within whose geographical zone they formerly resided. In other words, we have been endeavoring not only to gain new members but to retain all of our old ones—or find out the reason for their secession. All in all, our efforts in both directions have met with some degree of success.

We are happy to report as of today a *total of 613 members* in good standing as against 570 at this time last year or an *increase* over the corresponding period in 1938 of 8.0%. *Forty new active members and two new corporation memberships have been added this year, as against 38 active and four corporation memberships last year.* In addition, four members from previous years have been reinstated for a gain of 44 active members for the current year. A survey, as of the 6th of May last, shows the following standing as to membership in the local sections:

*In Active Membership*

8 sections with an increase  
2 sections no change  
2 sections with a decrease  
1 section not heard from.

*In Associate Membership*

8 sections with an increase  
1 section no change  
2 sections with a decrease  
2 sections not heard from.

As was called to your attention last year, we again find a few sections out of balance in the proportion of associate to active members. However, we know the officers of these sections are making every effort to bring about a better proportionate relationship in this respect. There are a number of them who feel that it is essential to the well-being of, and sustained interest in, their meetings to make use of a large associate membership, among whose ranks are many who actually would not qualify as, or do not wish to become, members of the national association. We appreciate and endorse that point of view.

The year 1939 promises to be a banner year in sustained growth both numerically and in the quality of membership attracted to our Association.

In conclusion, allow me once more to thank the members of the committee, particularly the chairmen of the local sections for their continued and effective co-operation during this past year. To them the major portion of credit should, for such a satisfactory showing, be extended.

### **The Membership Requirements Committee**

*R. C. SHERWOOD, Chairman*

At the annual meeting in Cincinnati, 1938, the Membership Requirements Committee reported its activities and suggested changes in the constitution. President Geddes reappointed the committee with instructions to draft proposed amendments to the constitution embodying the recommendations of the committee. This work was completed a few months ago, and reported to President Geddes. Under date of February 13, 1939, mimeographed copies of the proposed revisions were distributed to all active members of the Association for their consideration. The proposed revisions are made a part of this final report. The committee has now completed the work assigned to it.

(Editor's note: see secretary's minutes for report of action taken on proposed amendments.)

### **The Inter-Relations Committee**

*C. G. HARREL, Chairman*

Sometime during the early part of January, 1939, it was my privilege to be appointed to the chairmanship of the Inter-Relations Committee. Because of this rather late appointment, it has not been feasible during this short time to hold as many committee meetings or communicate with the committee as often as would be desired. In order that some definite program might be made, it has been necessary



for the chairman to start immediately certain work in regard to the issuing of invitations and programs. It is hoped that this will meet with the approval of the association as a whole, as well as the members of my committee.

In March, 1939, the Inter-Relations Committee, in cooperation with the Northwest Section of the American Association of Cereal Chemists, assisted in preparing a program which was held at the Leamington Hotel on March 31st. Copies of the printed program were sent to approximately 500 officials of the various milling and baking organizations throughout this country, as well as heads of educational institutions. Invitations to this program were likewise sent to members of our Northwest Section and to chairmen of local sections of the A. A. C. C.

On May 12, 1939, 270 letters were addressed to the executives of the milling and baking organizations in this country extending to them a most cordial invitation to be present at our meeting in Kansas City. Through the cooperation of the local committee of the Kansas City Section, we obtained a list of 163 high schools, junior colleges, and universities, which was carefully prepared by Dr. Wildish, of the Kansas City Junior College. These 163 educational institutions are located in and around Kansas City. A letter was written extending to them a cordial invitation to be present at one or more of our meetings which are now in progress. Along with the letter written to the industrial organizations and educational institutions, a copy of the preliminary report of the Program Committee, which appeared in Volume 47 of the News Letter issued in April, 1939, was attached.

I wish to report that our communications, which have been written since the first of the year, amount to approximately 1,000 letters.

### The Committee on Definitions of Technical Terms

QUICK LANDIS, *Chairman*

During the last year the committee has maintained a general survey of our field in order to recognize any serious ambiguity in terminology. We would like to call your attention to the recent publication of two dictionaries of scientific terms even though they may contain few of our own specialized terms, namely: *Standard Chemical and Technical Dictionary*, by H. Bennett, and *Dictionary of Scientific Terms*, by C. M. Beadnell.

It is the purpose of the committee to complete the collection of definitions of biometrical terms and to publish them under the authorship of C. L. Brooke, in a convenient form for ready reference.

If a sufficient demand should develop, it is believed that somewhat extended definitions for terms used in baking technology and cereal chemistry might profitably be undertaken. If these were so constructed as to appeal both to lay and technical individuals, a desirable unification of thought might be encouraged.

The circumstances under which this committee was first conceived have been, we believe, improved. Much of this improvement has arisen from extensive investigations and from the resulting advancement of scientific knowledge. We trust, however, that the efforts of the committee may have been of some assistance in this connection.

For the last few years our conventions have been singularly free from discussions arising from serious ambiguities in the use of technical terms. It therefore appears that the Committee has served its purpose and it is recommended that it be discharged.

### The History Committee

ROWLAND J. CLARK, *Chairman*

The History Committee wishes to express its deep appreciation to the Publicity Committee, to the sectional secretaries, the Association officers, and to the other individuals who have, during the past year, contributed to the historical files of our organization. Through the splendid cooperation of all these members, announcements of meetings, descriptions of programs, magazine clippings, together with other valuable historical records, have all been preserved in our organization's scrapbook.

Events become significant or fade into the mist of forgotten years in proportion to their influence on future trends of action. It is difficult to determine the true importance of an event until it is viewed in the light of past history. Therefore it is necessary for the History Committee to preserve all evidence and data pertaining to the Association's activities in order to evaluate rightly the course pursued.

Apparently cereal chemists stand higher in the esteem of the industry today than they did a year ago. There was a day when in times of depression the cereal chemist was the first to suffer. Now we have one of our leading milling executives stating in the columns of the News Letter: "The cereal chemist has been called to the rescue of the cereal-products industry." How welcome, yet how ludicrous, this statement would have sounded twenty-five years ago. Of course the cereal chemist has always been convinced of its truth; but it has taken time, patience, and hard toil to prove to the industry that chemical research holds the key to future progress. It seems that this recognition is an event that can be properly evaluated immediately because it partially reaches a goal toward which the Association has been striving for a quarter of a century.

The American Association of Cereal Chemists will this week render an accounting of its past year's work. History will be unfolded. The eyes of the industry are upon the actions of this gathering. The past twenty-five years are studded with vivid memories of accomplishments. The next twenty-five years can be made even greater.

### **The Traffic Committee**

G. NORMAN BRUCE, *Chairman*

The duties of the Traffic Committee are to list the most convenient routes to the national meeting, which were duly reported and listed in the News Letter. Experience on this duty for the past three years leads the present chairman to the opinion that the traffic arrangements are naturally a function of the convention arrangement program and should be handled by the convention committee through the chairman of each local section. The local chairman could appoint a traffic-wise member in each group to handle details and report to the convention committee for listing in the News Letter.

### **The Committee on Osborne Medal Award**

R. W. MITCHELL, *Chairman*

This committee functions somewhat differently from most committees of our society. While most of our committees strive to get work done with dispatch this committee is charged with the responsibility of going slowly. Our society has expressed a desire to have the Osborne medal represent its expression of tribute to very unusual accomplishment in the field of cereal chemistry.

During the past year the several names have been considered but the committee has chosen to move slowly so that time may permit a crystallization of sentiment. We come to you at this time to recommend that there be no award of the medal now, and that our action be reviewed by the committee during the coming year. In that way the Association will have the assurance afforded by the deliberations of two committee groups that the dignity of the award is being jealously guarded.

### **Report of the Secretary**

J. M. DOTY

The secretary has very little to report. The usual routine letters were written. A total of 2,423 carbon copies of letters written from May 1, 1938, to May 1, 1939, are now in his files.

### **The Committee on Resolutions**

WASHINGTON PLATT, *Chairman*

Whereas, the American Association of Cereal Chemists has again enjoyed a most successful year of activity and a profitable and enjoyable annual meeting; and

Whereas, this success has in large measure been due to the services so generously and efficiently rendered by the officers and committees of the Association,

Therefore be it resolved that the sincere appreciation of this Association be expressed to the officers who have served during the past year: President, W. F. Geddes; Vice-President, G. F. Garnatz; Secretary, J. M. Doty; Treasurer, Oscar Skovholt; and to the Program Committee, C. H. MacIntosh, Chairman; the Local Arrangement Committee, Perie Rumold, Chairman; The Ladies' Entertainment

Committee, Mrs. Perie Rumold, Chairman; and to all other committees who by their contributions have made the year's work so successful.

Be it further resolved that we express our appreciation for the services of M. J. Blish, Editor-in-Chief of *Cereal Chemistry*, and to R. M. Sandstedt, Managing Editor, and to their associates on the business and editorial staff of *Cereal Chemistry* for their excellent work during the year just passed.

Be it further resolved that we express our thanks to the Rev. David H. Owen, who delivered the invocation at our opening session.

Be it further resolved that we express our thanks to the Hon. Bryce B. Smith, Mayor of Kansas City, for the delightful talk and fine welcome which he extended to the Association on behalf of Kansas City.

Be it further resolved that we express our thanks and appreciation to those whose assistance and cooperation with the Local Arrangement Committee have contributed materially to the success of the many and varied features of our meeting namely:

To the following as donors of the shooting and golf prizes: Mr. Joe Hinckle of the Elliott Arms; Mr. O. L. Davis of Angler's Supply House; Mr. LeRoy Brouse of Gateway Sporting Goods; and Mr. R. J. Clark of Power and Light Co. (all of the foregoing being located in Kansas City, Missouri), American Agricultural Chemical Co., Anheuser-Busch, Inc., Durkee Famous Foods, Laboratory Construction Co., Monsanto Chemical Co., Precision Scientific Co., Standard Brands, Inc., Victor Chemical Co., and Wallace and Tiernan Co.

Be it further resolved that we express our thanks to the Manor Baking Co. for the handsomely decorated birthday cake symbolizing the twenty-fifth anniversary of the founding of this Association, and to the following firms for their hospitality in inviting the members of the Association for field inspection trips: Corn Products Refining Co., Goetz Brewing Co., Research Laboratory of Campbell-Taggart Research Corp., and Sheffield Seel Co.

Also to Mr. Adrian Hooper, Assistant Manager of the President Hotel, Kansas City, and to his staff and the Kansas City Chamber of Commerce.

And whereas the Association of Operative Millers will hold their annual meeting in Kansas City June 5th to 9th,

Be it resolved that we extend to them our best wishes that they may have a successful meeting and the expression of our anticipation of another year of continued cooperation between our two associations.

Whereas the American Society of Bakery Engineers generously granted to the American Association of Cereal Chemists a liberal portion of the program of their annual meeting and whereas Mr. William Hauck, President of the Society of Bakery Engineers, has kindly taken part in our own annual meeting,

Be it resolved that the Association renew its expression of the mutual value of the close cooperation and friendly spirit which has existed between these two organizations for so many years.

Be it resolved that the Association express its thanks to our guest speakers Dr. E. R. Weidlein, Dr. John H. Parker, and Dr. D. J. Hennesy, all of whom did so much to make our opening meeting an inspiration to cereal chemists.

Be it resolved that the Association express its thanks to Sergeant D. E. Bates and Mr. Francis M. O'Connor of the Kansas City Police Department for their exhibition of skill and courage in connection with our outing.

Be it resolved that the secretary be instructed to send a copy of an appropriate extract from these resolutions to each individual or organization named herein.

Be it resolved that we express our deep regret at the passing of loyal member A. D. Barbour, and be it further resolved that the Secretary be instructed to convey to his family the deep and heart-felt sympathies of the Association.

## BOOK REVIEW

**Durum Wheats and Their Utilization.** By Carl L. Alsberg. Volume 15, No. 7, of *Wheat Studies of the Food Research Institute*, Stanford University, California. 27 pages. Price 75 cents. April, 1939.

This publication presents in compact form an up-to-date picture of the world situation as regards durum wheats and durum wheat products. Origin, distribution, botanical features, and agronomic and cultural characteristics of durum wheats are indicated, and our present knowledge of the physical and biochemical properties of the grain itself is adequately set forth.

Going back to ancient times, all of the known methods of utilizing durum wheats are discussed, and the essential features of macaroni manufacture are described. Numerous and well chosen literature references are given for the benefit of those who may wish to consult original sources for more detailed information.

No one interested in durum wheat or in durum wheat technology can afford to be without a copy of this publication.

M. J. BLISH

## GLASS BLOWING MANUAL

The Laboratory and Pharmaceutical Division, Corning Glass Works, Corning, New York, has recently published a manual "Laboratory Glass Blowing with 'PYREX' brand Glass."

This manual is offered as an aid to the laboratory technician. It contains information on the working characteristics of "PYREX" brand Glass, an explanation of fundamental glass blowing operations, and recommendations of suitable tools and equipment. The manual will be supplied without charge, upon request, to users of Corning Glass Works' laboratory glassware.

# CEREAL CHEMISTRY

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No. 5

## ACTION OF BETA-AMYLASE FROM SOYBEANS ON VARIOUS STARCHES <sup>1</sup>

V. D. MARTIN, N. M. NAYLOR, and R. M. HIXON

Department of Chemistry, Iowa State College, Ames, Iowa

(Received for publication March 8, 1939)

Previous work (Martin and Newton, 1938) has shown that when starches from different sources are heated at their optimum gelatinization temperatures and then cooled, beta-amylase action takes place at the same rate on all the starches. Since the series studied contained starches with both high and low phosphorus and fatty-acid content, the question of the importance of these groups in enzyme action arose. Furthermore, the appearance of a flocculent material in the digestion mixtures and the fact that the maltose formed accounted for only about 70% of the starch showed the presence of some interesting residual substances.

Mÿrback (1937) suggested that both the fatty acids and the phosphorus in starches might stop the enzyme action. Samec (1927) concluded that the phosphorus present in potato starch (0.015%) inhibits or blocks the action of the enzyme. On the other hand, Pringsheim and Ginsberg (1935) reported that complete hydrolysis of starch was obtained without liberating any free phosphoric acid. Taylor and Sherman (1933) concluded that lipase-free amylase could attack the linkages of fatty acids to the starch molecules. However, since Schoch (1938) was able to remove the fatty acids from corn starch by extraction methods, it is doubtful if a strictly chemical bond links the fatty acids to the starch.

The fact that a flocculent material appears in the enzyme digestion of starch was first noticed by Baker (1902). Other investigators (Fernbach and Wolff, 1904; Sallinger, 1919; Schryver and Thomas, 1923; Ling and Nanji, 1925; Hermano and Rask, 1926; Malloch, 1929; and Clayson and Schryver, 1923) have described the appearance of this material in amylase digestions. Sherman and Punnett (1916)

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filtered out the flocculent material and weighed it. They found that the amount (1.08–1.4%) was independent of the enzyme used.

Baker (1902), Syniewski (1925), Freeman and Hopkins (1936), Blom, Bak, and Braae (1936), and Hanes (1937) have shown that the increase in reducing action during the beta-amylase digestion of potato starch is due almost entirely to the formation of maltose. The limit of production of maltose is 60%–67% of the starch as confirmed by van Klinkenberg (1932), Samec (1935) and Hanes (1937). Work in this laboratory has shown that the same limit is reached in digestions of corn, wheat, rice, and tapioca starches as well.

Throughout the hydrolysis of starch by beta-amylase, residual starch-like substances are present which can be precipitated by 50%–60% alcohol. The material that is left at the end of the reaction, which should amount to 30%–35% of the starch on the basis of the maltose calculation, was separated by Wijsman (1890), who named it "erythrogranulose." Baker (1902) and Haworth, Hirst and Waine (1935) prepared it in about the same way and named it "alpha amylodextrin." A summary of the properties of this material as obtained by different investigators is given by Hanes (1937). The results are not at all in agreement.

That this material is a part of the original starch which the beta-amylase cannot attack seems more probable than that it is a product of a secondary reaction. Pringsheim and Beiser (1924) concluded that the 60% alcohol precipitate from beta-amylase digestions was an intact part of the original starch. Mjörback (1937) considered that the enzyme could split maltose from all starch molecules but to varying degrees. The residual material would then consist of fragments of the original starch molecules which for some reason cannot be attacked by the amylase.

From the point of view of Mjörback (1937) the variations in the reported properties of this material could be due to differences in the starches. On the basis of this concept the residual material from beta-amylase action on the different starches should contain that portion of the starch molecule which causes the difference in the various properties of the starches.

### Preparation of Residual Materials

The general procedure followed in separating the residual materials from the beta-amylase digestions of different starches can be represented by Figure 1. Precipitates A and B were prepared from corn, wheat, rice, potato, and tapioca starches. The amylase was prepared from soybean meal by the method of Newton and Naylor (1939), who classified the enzyme as beta amylase from mutarotation studies.

The starches were gelatinized at the temperature found to be optimum for beta-amylase action (Martin and Newton, 1938). The substrates were prepared by the following method. Eight liters of a solution containing 980 cc. of 0.2M  $\text{NaH}_2\text{PO}_4$  and 20 cc. of 0.2M  $\text{Na}_2\text{HPO}_4$  was placed in a 16-liter balloon flask in a water bath and allowed to come to the temperature desired. Then 600 g. of untreated starch, suspended in 2 liters of water, was poured into the flask with stirring. After 30 minutes the flask was removed from the bath and cooled in running water, and placed in a thermostat at 40°C. One hundred cc. of a suspension containing 700 mg. of soybean amylase was added. The mixture was stirred vigorously and let stand 5

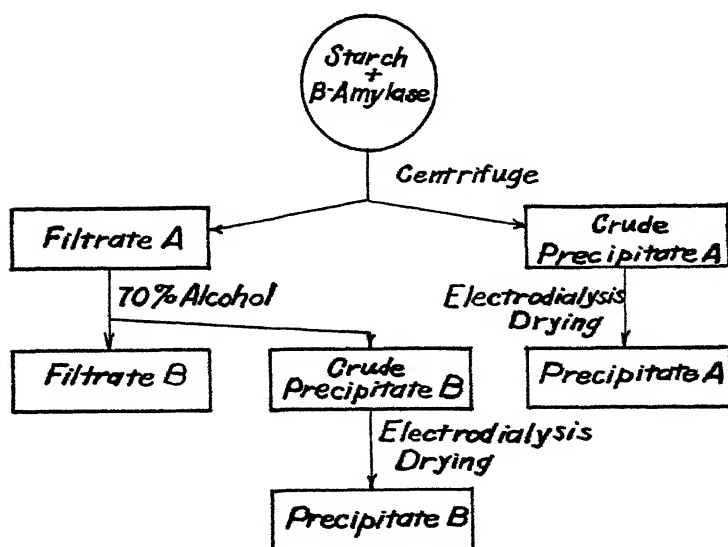


Fig. 1. Procedure followed in separating the residual materials from beta-amylase digestions of different starches.

hours. At the end of this time the flask was placed in a refrigerator for 16 hours. This procedure seemed to facilitate the removal of precipitate A. The digestion mixture was then run through a Sharples supercentrifuge. One-cc. samples were removed from the centrifugate for the sugar determination.

The solid material (precipitate A) which collected in the bowl of the centrifuge was shaken up in 1 liter of water. This suspension was electrodyalyzed because the material could not be recovered from the wash water by supercentrifuging. When the material had settled in the dialyzer (after about 12 hours) the supernatant liquor was siphoned off. More water was added, the solids shaken up, and



dialysis continued. This process was repeated two or three times until the liquid from the anode chamber gave no test for phosphate.

Attempts to dry the precipitate in air at this point resulted in a dark-colored hard mass which could not be ground. Therefore, after dialysis the thick suspension from the bottom of the dialyzer was put into twice its volume of absolute alcohol and allowed to stand overnight. The supernatant liquid was siphoned off and another portion of absolute alcohol added. The solid material was again allowed to settle. This treatment was continued until the precipitate was sufficiently granular to filter with suction. Dehydration was completed by grinding under several portions of absolute alcohol. The material was then dried with ether and placed in a vacuum desiccator for two or three days. When dry, precipitate A was ground to a white powder in an agate mortar.

The fraction called precipitate B (Fig. 1) was prepared from five different starches. The precipitate was prepared by adding 2200 cc. of absolute alcohol to 1500 cc. of the centrifugate from the preparation of precipitate A. The mixture was allowed to settle and then centrifuged or the supernatant liquid siphoned off, depending on the nature of the precipitate. The appearance and nature of precipitate B were quite different from the cereal and root starches. When prepared from the cereal starches, it was curdy and settled out nicely, while if prepared from the root starches, it was formed as a transparent sticky mass. When the precipitate was flocculent, it was washed once with 60% alcohol, then dried by grinding under absolute alcohol. The gummy precipitates were repeatedly ground under absolute alcohol and dried to white powders.

These crude products were later redissolved in water and electro-dialyzed until free from phosphate. The material was recovered as before. However, in the case of the material from potato and tapioca starches, electrodialysis was necessary to separate it from the alcohol mixtures. In this purification process, 50% to 75% of precipitate B was lost.

The yields of precipitates A and B from a series of starches are given in Table I. The yields of precipitate B are based on the amount of the crude product. The percentage of maltose formed is also given and is based directly on the total reducing value of the solution.

### Characterization of Precipitates

The precipitates were characterized as to further hydrolysis by fresh beta-amylase, by phosphorus and fatty-acid content, by reducing power, and by iodine precipitation according to the procedure of Denny (1922). The results are recorded in Table II.

TABLE I  
YIELDS OF SOME PRODUCTS OF BETA-AMYLASE ACTION

Kind of starch	Maltose equiv.	Precipitate A	Precipitate B	Total yields
	$\zeta_c$	$\zeta_c$	$\zeta_c$	$\zeta_c$
Corn	51.4	1.64	32.2	88.24
Rice	38.5	1.92	34.4	74.82
Wheat	51.8	1.0	38.8	91.6
Potato	55.1	0.84	30.0	85.94
Tapioca	55.6	0.05	33.5	89.15

TABLE II  
PROPERTIES OF PRODUCTS OF BETA-AMYLASE ACTION

Substance	Per- cent yield	Enzy- matic dige- stion, $\zeta_c$ malt- ose	Reducing power		Phosphorus		Fat		Starch <sup>4</sup>	
			Mg. malt- ose/g	Reu <sup>1</sup>	$\zeta_c$ <sup>2</sup>	Re- covery <sup>3</sup>	$\zeta_c$ <sup>2</sup>	Re- covery <sup>3</sup>	Mg. I ads	$\zeta_c$ Stch
Corn starch	—	58.5	16.8	5.5	0.015	—	0.66	—	2.9	65.6
Ppt. A	1.64	14.1	27.3	9.6	0.031	3.38	1.31	3.26	3.25	69.0
Ppt. B	32.2	15.3	27.3	8.2	0.033	70.8	0.71	34.5	0.86	19.6
Rice starch	—	65.7	37.8	7.8	0.035	—	0.62	—	3.12	71.0
Ppt. A	1.92	30.0	48.3	11.2	0.041	2.25	0.95	2.90	3.9	89.2
Ppt. B	34.4	34.0	48.3	13.0	0.033	32.8	0.56	31.0	4.05	92.0
Wheat starch	—	60.0	25.2	10.4	0.051	—	0.57	—	3.15	71.6
Ppt. A	1.0	23.9	46.2	11.3	0.041	1.8	0.90	1.5	3.2	76.7
Ppt. B	38.8	26.2	46.2	13.0	0.152	103.0	0.91	51.4	4.0	90.5
Potato starch	—	59.3	16.8	4.3	0.050	—	0.076	—	3.5	79.5
Ppt. A	0.84	9.65	63.0	36.5	0.112	1.88	0.17	1.8	—	—
Ppt. B	30.0	9.02	63.0	13.8	0.222	102.5	0.18	71.0	—	—
Tapioca starch	—	77.2	33.6	4.3	0.010	—	0.174	—	2.95	67.0
Ppt. A	0.05	6.3	52.5	19.0	0.020	1.0	0.51	1.46	—	—
Ppt. B	33.5	9.8	33.6	14.6	0.020	67.0	0.22	42.3	—	—

<sup>1</sup> Reu determinations were made by B. Brimhall.

<sup>2</sup> Actual percentage present.

<sup>3</sup> The recovery of fat and phosphorus is expressed as percentage of the groups in the original starch and is based on the yields given in the first column.

<sup>4</sup> The adsorbed iodine method for determining starch according to Denny (1922).

For the further beta-amylase action, one-percent unbuffered substrates were used. The substrates were boiled. Five cc. of a suspension containing 40 mg. of soybean amylase in 50 cc. of water was added to 100 cc. of substrate. Five-cc. samples were removed at intervals for sugar determination. The original starches were tested in exactly the same way as the preparations.

Since the phosphorus content of these materials was very low, micro technique was used in analyzing for phosphorus. The volu-

metric method of Pregl (1937) was modified somewhat, in that the yellow precipitate was washed with 3% potassium-nitrate solution instead of alcohol. The solutions used were the same as in the Pregl method. The fatty-acid content of the original starches and the precipitates was determined by the method of Taylor and Nelson (1920). In the columns headed "recovery" in Table II under fat and phosphorus, the amount of these groups present in the preparations is expressed as percentage of these groups present in the original starches. The calculated recovery of fat and phosphorus is based on the yields given in the first column.

The reducing power of the preparations was measured both against ferricyanide (Martin and Newton, 1938) and against copper by the method of Farrow (Richardson, Higgenbotham, and Farrow, 1936). The determinations by the potentiometric method were made on 5.0 cc. of a one-percent suspension that had been boiled. The reducing value was calculated as mg. of maltose per gram of sample. The reducing power against copper is given as  $R_{cu}$  values according to Farrow.

The adsorbed-iodine method for the determination of starch (Denny, 1922) was used to compare the precipitates A and B with the original starches. The calculated percentage recovery is based on the factor  $(g. \text{ starch}) / (g. \text{ iodine}) = 0.11$  (Denny, 1922) determined on soluble potato starch. The preparations from potato and tapioca starches gave the customary deep violet-black color, but no precipitate could be centrifuged or filtered out.

### Discussion and Conclusions

The data summarized in Table II show that the precipitates are quite different from the original starches. The reducing power and phosphorus and fatty-acid contents are, in general, higher than in the original starches. This corresponds to the theory that these materials are fragments of the starch molecules and contain a concentration of the associated phosphorus and fatty-acid groups. The apparently anomalous results on the original starches with the starch determination of Denny are due to the use of the factor determined on soluble starch. The method is apparently not specific for unchanged starch.

If fractions A and B from each individual starch are compared, there appears to be no consistent difference in the chemical properties measured. The reducing power of precipitates A and B is very nearly the same, by either the potentiometric method or the copper method of Farrow. The fat content is slightly higher in precipitate A from corn, rice, and tapioca starches. The phosphorus content of precipitates A and B is about the same except for wheat and potato

starches, where precipitate B is much higher in phosphorus. In the starch determination the behavior of precipitates A and B from any one kind of starch is very similar. The only consistent difference between the two products from any one kind of starch is the fact that precipitate A flocculates directly from the digestion, while precipitate B comes out in 70% alcohol.

The differences in the original starches, particularly between the cereal and root starches, are magnified in the residual material from beta-amylase action. The reducing power of the residual materials from potato and tapioca starches is higher than of these precipitates from the cereal starches, but the residual materials from the root starches are not hydrolyzed as far by fresh beta-amylase. The fatty-acid contents of the precipitates from the digestions of root starches are lower than those from the cereal starches, but the difference between the residues from potato and from tapioca starches appears larger than that between the cereal and root starches. There are no significant trends in the phosphorus content. The starch determinations show a marked difference, which is probably due to the difference in physical nature of these materials as noted in their preparation. The differences in the residual materials from cereal and root starches are apparently of degree rather than kind and cannot be entirely explained on the basis of the phosphorus and fatty-acid content.

The role of the phosphorus and fatty acids is apparently unimportant in the beta-amylase digestion of starches. While the optimum temperatures for preparing the substrates may be correlated to the fatty-acid content, the importance of the phosphorus is much more obscure. When the different starches are prepared at their optimum temperatures, the rate of beta-amylase action is the same, and the digestion limits are about the same. Therefore, the blocking of the enzyme does not seem to be caused by the presence of these groups.

### Summary

The non-digested portions of corn, wheat, rice, potato, and tapioca starches resulting from the action of soybean beta-amylase were used to prepare a flocculent, water-insoluble fraction and a gummy, water-soluble fraction precipitated by 70% alcohol.

These fractions have been characterized as to reducing power, further hydrolysis by beta-amylase, phosphorus and fatty-acid content, and precipitation by iodine according to the procedure of Denny.

The flocculent material and the alcohol precipitate from any one kind of starch are apparently very similar.

The physical natures of these preparations are very different, depending on whether they originate from cereal or root starches.

The phosphorus and fatty-acid groups do not appear to be the agents which block the action of beta amylase at 60%-70% conversion of starch to maltose.

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## A NOTE ON MOISTURE INTERCHANGE IN MIXED WHEATS, WITH OBSERVATIONS ON THE RATE OF ABSORPTION OF MOISTURE BY WHEAT

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The observations recorded in this note were made in preliminary work in the large field of wheat conditioning and the moisture relationships of wheats. It is a common practice to partially dry excessively damp wheat by mixing it with a dry wheat and allowing the mixture to lie for a convenient length of time. It is well known that under such conditions the wheats tend to equalise in moisture content, and, if the wheats are suitably chosen, the resulting mixture may be in quite good condition for milling. Little, however, is known precisely as to the nature and extent of the process of moisture interchange involved.

The work here reported was done mainly on Karachi and English wheats—which are sometimes treated in this way by English millers. In the first experiment three quarters of an approximately 50-50 blend of English and mixed Karachi wheats, which had “natural” moisture

contents before mixing of 20.65% and 10.10% respectively, were allowed to remain in a three-quarter wooden zinc-lined bin for about six weeks. From time to time, daily at first, then at less frequent intervals, samples were taken, the grains of each of the two wheats were separated by hand picking, and moisture determinations made on each lot (results shown in Table I and Figure 1). It will be noticed

TABLE I  
PRELIMINARY OBSERVATIONS ON MOISTURE INTERCHANGE IN WHEAT DURING STORAGE

Time in days from start	Moisture content of English wheat	Moisture content of Karachi wheat	Difference
	%	%	%
0	20.65 (before mixing)	10.10 (before mixing)	10.55
1	17.82	13.18	4.64
2	17.02	13.74	3.28
3	17.13	14.71	2.42
4	16.72	13.90	2.82
5	15.74	13.62	2.12
6	16.65	14.38	2.27
7	17.14	14.27	2.87
8	17.20	14.32	2.88
9	17.20	14.47	2.73
13	17.10	15.29	1.81
14	17.02	14.43	2.59
15	16.96	14.36	2.60
16	16.88	14.29	2.59
19	17.05	14.70	2.35
20	16.92	14.48	2.44
27	16.83	14.38	2.45
29	16.97	14.59	2.38
33	16.98	14.56	2.42
35	16.75	14.45	2.30
40	17.10	14.79	2.31
43	16.91	14.64	2.27
47	17.00	14.80	2.20
Mean of last 17 determinations	16.98	14.54	2.44

Mean moisture of unmixed wheats, 15.38%.

Mean of means of last 17 determinations, 15.76%.

that moisture interchange between the damp and dry wheats was very rapid during the first two days, while practically no change occurred after six days. From the third until the 47th day after mixing an approximately constant difference in moisture content of 2.4% persisted between the two wheats, and there appeared to be no tendency for this difference to decrease.

The results, striking as they are, are obviously subject to certain disturbing factors. In the first place, the mixing of the wheats had been done on the commercial scale by the power-driven mixers at the foot of the storage bins in a commercial mill, and there was no cer-

tainty therefore that the mixture was either accurately 50-50 or uniform. Again, under the conditions of storage, the mixture was likely to be affected by change in atmospheric conditions, although as shown by Table I, this effect was actually very small. The ready response of wheat to change in relative humidity is, however, well known.

Further tests were made under more precise conditions. A sample of red English wheat with a "natural" moisture content of 17.33% was sprayed evenly (to increase its moisture content) and allowed to stand several days. Immediately before the experiments its moisture content was 21.99%. In order to make the separation (by hand picking) of the mixtures to be tested easy and quick, white Karachi grains only were used. The white grains were separated by hand

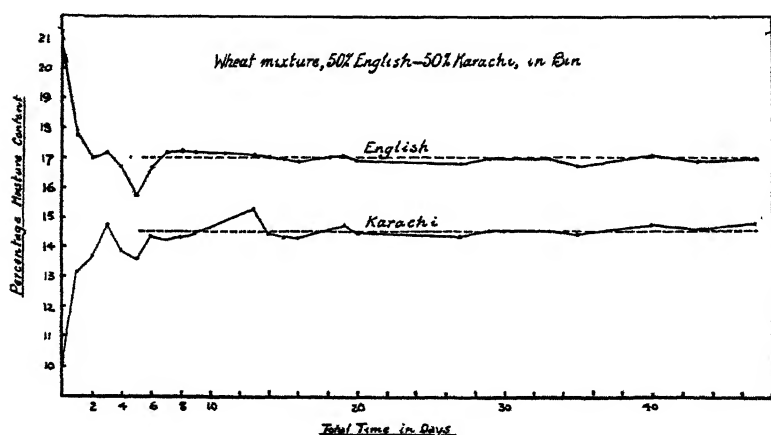


Figure 1

picking from a considerable sample of the original Karachi and were easily picked out after mixing with the red English grains. Immediately before use in the tests the moisture content of this well-mixed white Karachi was found to be 12.62%.

Several glass bulbs each capable of holding over 30 grams of wheat were blown and provided with necks through which wheat could be introduced; the necks were shaped to allow convenient sealing-off in the blow-pipe flame.

Fifteen grams of the English wheat was thoroughly mixed with 15 grams of the Karachi, and the mixture introduced into a glass bulb, which was then sealed in the blow-pipe flame.

Twelve bulbs were so prepared and immersed in a water bath, whose temperature was maintained at 20° C. At regular intervals of



time a bulb was removed, opened, the mixture separated, and moisture determinations made on the two components. The separation of the mixture by hand picking did not take longer than three or four minutes. The results are given in Table II and Figure 2.

TABLE II

MOISTURE INTERCHANGE IN A 50-50 MIXTURE OF DAMP ENGLISH AND DRY WHITE KARACHI WHEATS

Time in days from start	Moisture of English	Moisture of Karachi	Difference
	%	%	%
0 (before mixing)	21.99	12.62	9.37
1	18.49	15.54	2.95
2	18.09	16.32	1.77
3	18.05	16.53	1.42
4	17.86	16.34	1.52
5	18.02	16.63	1.39
7	18.02	16.91	1.11
9	17.87	16.82	1.05
11	18.00	16.99	1.01
17	17.81	16.59	1.22
21	17.98	16.93	1.05
25	17.75	16.73	1.02
Mean of last 7 determinations	17.92	16.80	1.12

Mean moisture of unmixed wheats, 17.31%.  
Mean of means of last 7 determinations, 17.36%.

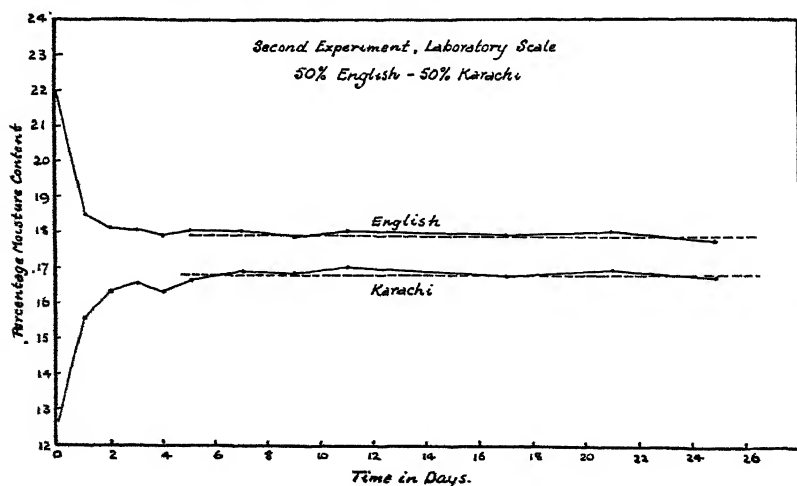


Figure 2

Along with the above a second series of tests was run under similar conditions except that the mixtures consisted of English wheat which had been dried and white Karachi which had been moistened. The wheats were initially the same as those used for the last series of tests,

but several days before the tests the English was dried at 50° C. and the Karachi moistened evenly by spraying. At the time of the tests the moisture contents of the well-mixed wheats were: English, 11.36%; Karachi, 26.56%. The results are given in Table III and Figure 3.

TABLE III  
MOISTURE INTERCHANGE IN A 50-50 MIXTURE OF DRY ENGLISH AND DAMP  
WHITE KARACHI WHEATS

Time in days from start	Moisture of English	Moisture of Karachi	Difference
	%	%	%
0 (before mixing)	11.36	26.56	15.20
1	17.69	21.58	3.89
2	18.93	20.41	1.48
3	19.19	20.14	0.95
4	19.20	19.94	0.74
5	19.25	19.98	0.73
7	19.62	20.08	0.46
9	19.42	20.47	1.05
11	19.47	19.93	0.46
17	19.40	19.83	0.43
21	19.52	20.01	0.49
25	19.29	19.72	0.43
Mean of last 7 determinations	19.42	20.00	0.58

Mean moisture of unmixed wheats, 18.96%.

Mean of means of last 7 determinations, 19.71%.

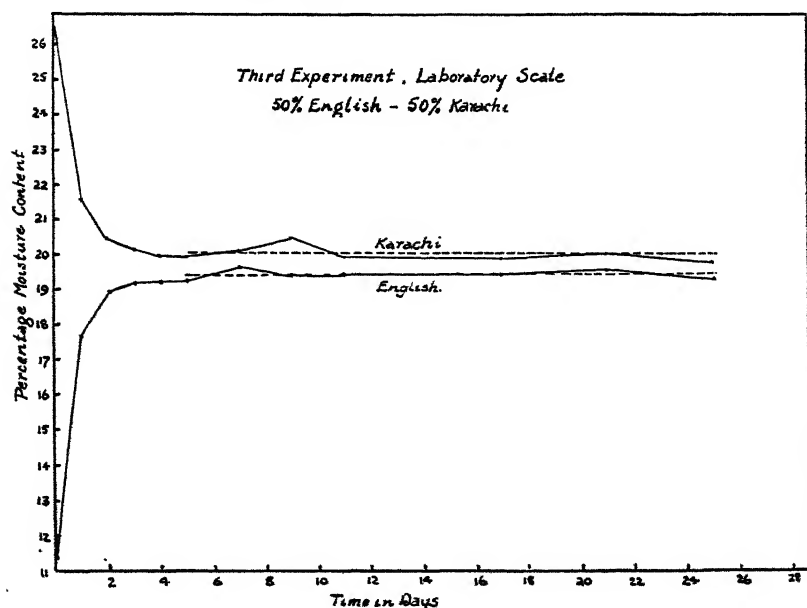


Figure 3

The results recorded are of considerable interest. In all three experiments the change in moisture content was very rapid for the first day and relatively small during the second and third days. After three days no significant change in moisture content occurred. In the large-scale experiment conducted first, the English wheat retained approximately 2.4% more moisture than the Karachi after 45 days; in the second experiment, carried out under more strictly uniform conditions, another and different sample of English wheat retained 1.12% more moisture than Karachi, with no sign of further change during 23 days; in the third experiment, in which Karachi was the damp wheat and English the dry, the Karachi retained 0.58% more moisture than the English, with no sign of change during 22 days. The general conclusions emerge that (1) when wheats of different moisture contents are mixed, a rapid interchange of moisture occurs at first but exact equalisation of moisture content never takes place; (2) however long the mixture is allowed to lie, the damper of the two wheats will permanently remain slightly damper than the originally drier wheat, quite irrespective of whether the damper wheat is soft and starchy, such as English, or hard and vitreous like Indian. This phenomenon is not confined to wheat, but is well known in connection with other colloidal materials. It was observed with silica gel many years ago by van Bemmelen, with wool by W. D. Hartshorne (1918), and with cotton by Orme Masson (1906); while extensive quantitative studies of the phenomenon have been made in connection with silica gel by Zsigmondy, Anderson (1914), and others, and in connection with cotton by Urquhart and Williams (1926).

#### Rate of Absorption of Water Vapour by Wheat

The point of most interest to millers is the great speed with which the transference of moisture takes place during the first day. Every berry is in actual contact with several other berries at a number of points and some moisture may pass from the damper to the drier berries through these points of contact. It is more probable, however (and there is some evidence to support the view), that the moisture transference takes place via the air. The tables show that the drier wheat gained from 3% to 5% of moisture in the first day and  $\frac{1}{2}\%$  to  $1\frac{1}{4}\%$  the second, the actual amounts absorbed depending on the initial difference in moisture contents between the two wheats. The change was hardly significant after two days and entirely insignificant after three days. It is interesting that where cold conditioning is the rule, as in U. S. A., the wheat is rarely allowed to lie more than three days, and it is probable that two days are adequate for any wheat. In the light of the above results, it is possible that the well-known

difficulty of getting sufficient moisture into hard, dry wheats such as durum or Indians is due not to the hardness but rather to the dryness of the wheat. Water penetrates a hard wheat quite rapidly, but in raising durums or Indians from, say, 8% or 10% to 16% or 17% moisture it is impossible for the wheat, after whizzing, to retain sufficient moisture in the wet film on the surface to raise the moisture content to the desired amount. Hence the wheat has to be washed and whizzed several times at intervals.

To investigate the matter further, the experiments were repeated with a mixture of 75% English (moisture content 21.39%) and 25% durum (moisture content 9.74%), and moisture determinations were made every two hours for the first 24 hours and then at longer intervals (Table IV and Figure 4). A shorter series was also carried out with a 50-50 mixture of English and durum wheats. All samples were kept in sealed tubes or bottles in a water bath at 20° C.

TABLE IV  
MOISTURE INTERCHANGE IN MIXED ENGLISH AND DURUM WHEATS

Time	75% English + 25% durum—moisture content (%)		50% English + 50% durum—moisture content (%)	
	Durum	English	Durum	English
Unmixed wheats at start . . .	9.74	21.39	9.74	21.39
2 hours . . . . .	13.06	19.95	11.39	18.53
4 hours . . . . .	13.70	19.58	12.04	18.11
6 hours . . . . .	14.36	19.15	—	—
8 hours . . . . .	15.18	19.37	12.98	17.99
10 hours . . . . .	15.07	19.33	—	—
12 hours . . . . .	15.23	19.12	—	—
14 hours . . . . .	15.86	19.14	—	—
16 hours . . . . .	15.95	19.04	—	—
18 hours . . . . .	16.17	18.83	—	—
21 hours . . . . .	16.48	18.90	—	—
24 hours . . . . .	16.39	18.83	13.97	17.04
2 days . . . . .	17.23	18.70	14.49	16.86
3 days . . . . .	17.37	18.80	14.73	16.85
4 days . . . . .	17.51	18.70	14.61	16.51
6 days . . . . .	17.49	18.72	14.61	16.50
26 days . . . . .	17.58	18.64	—	—
29 days . . . . .	17.55	18.62	14.76	16.51
3½ years . . . . .	17.65	18.24	14.88	16.08
Mean moisture content after 3 days . . . . .	17.53	18.67	14.66	16.51
Mean difference after 3 days . . . .	1.14%		1.85%	

The rapidity with which moisture was absorbed by the dry durum wheat in the first few hours of the experiment is shown in Table IV and Figure 4. The moisture content of the durum in the 75% English and 25% durum mixture increased from 9.74% to 13.06% in the first

two hours, *i.e.*, an increase of 3.32% on the ordinary air-dry basis. In 8 hours the increase was 5.44% and in 24 hours it was 6.65%.<sup>1</sup> In other words, the amount absorbed in the first two hours was about equal to that absorbed in the next 22 hours. Very little change occurred after two days, and none at all after three days.

In order to compare directly the relative absorbing rates of durum and English wheats, a technique different from that described above was adopted. Circular aluminium pans  $2\frac{3}{4}$  inches in diameter by  $\frac{1}{2}$  inch deep were used as containers. Sufficient durum or English wheat (about 8 g.) was used to cover the bottom of the pan, *i.e.*, the layer of wheat was one berry deep. The separate wheats were placed

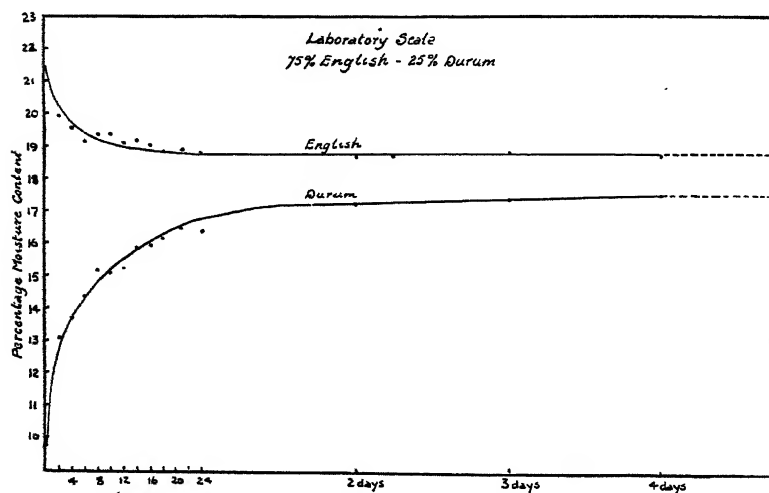


Figure 4

in the pans, which were placed in closed glass vessels of special design which contained 20% sulphuric acid, which is in equilibrium with an atmosphere of 87% relative humidity. The air was not kept mechanically in motion although gentle air mixing must have occurred through convection due to the relatively small temperature differences between the acid and the chamber covers. It is possible that the actual relative humidity near the wheat was lower than that near the acid, and that the "stationary air film" around the wheat was of greater thickness than with wheat in a moving air stream (Lewis, 1922).

Both these factors would lower rate of absorption, and we know from other observations of our own that the rate of absorption under these conditions is actually lower than that observed when wheat of these cond.

<sup>1</sup> Calculated as percentages of the dry matter of the wheat, these three figures become 4.22%, 7.10%, and 8.81% respectively.

similar moisture content is treated with an air stream of 87% humidity. The experimental conditions however were such that the acid provided a regulated constant source of supply of water vapour upon which the two samples of wheat could draw quite comparably. The actual rates of absorption are not material to the discussion; only the relative rates under the particular experimental conditions are of interest. The apparatus was so arranged that the aluminium containers with their wheats could be weighed at 15-minute intervals without removing them from their positions, *i.e.*, without interfering in any way with the absorption process. For a detailed description of the apparatus and method of work see E. A. Fisher (1927) and B. A. Keen (1914). The temperature throughout the experiment was 12.5° to 14° C. In these experiments the moisture contents are calculated on the dry weight basis, so that the results are more easily and strictly comparable among themselves. The results are summarized graphically in Figure 5.

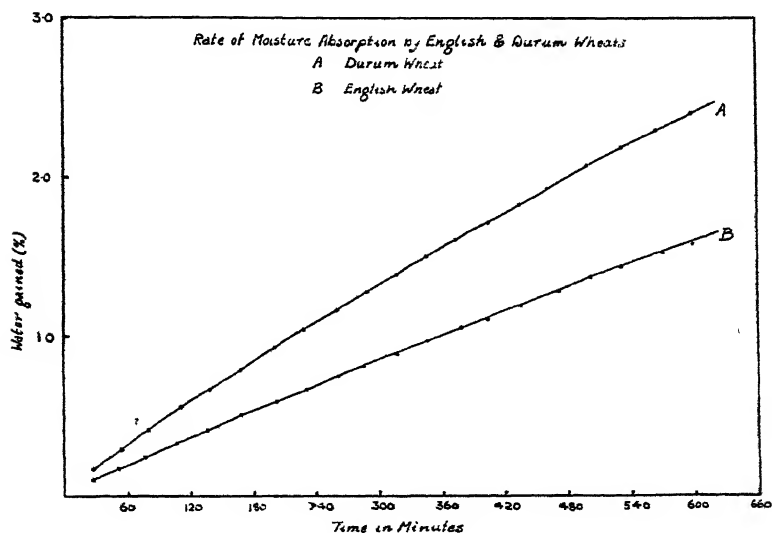


Figure 5

The conditions in this series of experiments were entirely different from those of the earlier series. With the mixed wheats the dry obtained moisture from the damp; the two processes therefore slowed up rapidly as the difference in moisture contents diminished. In these later experiments the wheats were absorbing water vapour from a comparatively large reservoir (the dilute sulphuric acid via the air) of constant vapour pressure. The absorption of moisture therefore proceeded at an almost constant rate; it slowed up very gradually as the

moisture content of the wheat increased. Figure 5 shows that the durum wheat absorbed water vapour approximately 50% faster than the English. The moisture contents, on a dry weight basis, at the commencement were durum 10.79%, English 14.44%; after 10 hours the moisture contents were durum 13.19%, English 16.02%. The average moisture contents throughout the experiment were therefore durum 11.99%, English 15.23%.

It is known that *over a small range*, other conditions being equal, the rate of absorption is approximately inversely proportional to the moisture contents. To make a strict comparison, therefore, of the two rates, the absorption (during 10 hours) of the English (1.578%) should be multiplied by its average moisture content (15.23%) and the product divided by the average moisture content of the durum (11.99%); *i.e.*,  $1.578 \times 15.23 / 11.99 = 2.004\%$ , which should equal the absorption (during 10 hours) of the durum wheat. The observed absorption was 2.405%, which was approximately 20% higher than that of the English.

It appears therefore that under strictly comparable conditions of moisture content, atmospheric humidity, and temperature the hard and vitreous durum wheat will absorb water vapour considerably more rapidly than will soft and starchy English wheat.

Two other considerations must be taken into account in a detailed study of this problem: (1) Durum grains are smaller than English; in equal bulks (weights or volumes) of these two wheats there will be more durum grains than English; the durum therefore will possess the larger collecting surface and this factor may be sufficient to account for the observed differences in absorption rates. (2) It has not been demonstrated that water absorbed as *vapour* from the atmosphere passes through the berry at the same rate or in the same manner as liquid water absorbed from a wet film covering the berry such as exists after washing and whizzing.

### Summary

It is a common practice to partially dry excessively damp wheat by mixing it with a dry wheat and allowing the mixture to lie for a convenient length of time. It is well known that under such conditions the wheats tend to equalize in moisture content, but little is known concerning the nature and extent of the process of moisture interchange involved. The problem has been studied on both a laboratory scale and a semi-commercial scale and it has been shown that (1) when wheats of different moisture contents are mixed a rapid interchange of moisture occurs at first but exact equalization of moisture content never takes place; (2) however long the mixture is allowed to lie the

damper of the two wheats will permanently remain slightly damper than the originally drier wheat, quite irrespective of whether the damper wheat is soft and starchy, such as English, or hard and vitreous, like Indian or durum.

The speed with which wheat will absorb moisture may be surprisingly great. In a mixture of 75% damp English (moisture 21.39%) and 25% dry durum (moisture 9.74%) the moisture taken up by the durum in the first two hours was almost equal to that absorbed in the next 22 hours. Very little change in moisture content occurs after two days, and the change is entirely insignificant after three days.

The evidence suggests that the well-known difficulty of getting sufficient water into hard, dry wheats such as durums and Indians is due not to the hardness but to the dryness of the wheat. To test this point the rate of absorption of water vapour from the air by durum and by English wheat was measured, and it was found that under strictly similar conditions of temperature, atmospheric humidity, and moisture content, durum wheat may absorb moisture at a rate 20% faster than soft and starchy English wheat.

#### Acknowledgment

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# OBSERVATIONS ON THE RATE OF MOVEMENT OF WATER IN WHEAT

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(Received for publication May 12, 1939)

In an earlier paper (Fisher and Jones, 1939) it was shown that when wheats of different moisture contents are mixed a rapid interchange of moisture occurs. The initial rate of interchange may be surprisingly great if the difference in moisture content is considerable. In a mixture of 75% damp English wheat (moisture content 21.39%) and 25% dry durum (moisture content 9.74%) the moisture taken up by the durum in the first two hours (3.3%) was equal to that absorbed in the next 22 hours. Very little change in moisture content occurred after two days, and the change was entirely insignificant after three days.

The evidence suggested that the well known difficulty of getting sufficient water into hard dry wheats such as durums and Indians is due, not to the hardness, but to the dryness of the wheats. It was found that, under strictly comparable conditions of temperature, atmospheric humidity, and moisture content, durum wheat may absorb moisture at a rate 20% faster than soft and starchy wheat.

It was pointed out that two other considerations must be taken into account in a detailed study of this problem: (1) Durum grains are smaller than English. In equal bulks (weights or volumes) of these two wheats there will be more durum grains than English. The durum, therefore, will possess the larger collecting surface and this factor may be sufficient to account for the observed differences in absorption rates. (2) It has not been demonstrated that water absorbed as *vapour* from the atmosphere passes through the berry at the same rate or in the same manner as liquid water absorbed from a wet film covering the berry such as exists after washing and whizzing.

The whole problem of moisture absorption by wheat is of importance in flour milling technology and further attempts have been made to obtain information that would throw light on the real nature of the process.

One line of study that has been followed is based on the volume changes that must take place when wheat and water are mixed and allowed to remain undisturbed at constant temperature. It is well known that when dry wool, cotton, gelatin, wheat, or flour is mixed with water heat is evolved as indicated by a rise in temperature. The drier the wool or the wheat the greater is the heat evolution.

This heat production is an indication of some kind of union, which may be either chemical or physical, between the solid material and at least some of the water. The phenomenon is quite general. When a sheet of glass is wetted by water some kind of combination occurs between the glass and some of the water as shown by the fact that the glass cannot be *shaken* dry; a film of water sticks to the glass. During the wetting heat is given out, but owing to the small size of the surface the heat production is so small that it can be detected only by means of specially designed apparatus. Very finely powdered glass would show a higher *heat of wetting* per unit weight than sheet glass on account of the greater surface. All colloidal materials show this phenomenon to a relatively great extent on account of their enormous surfaces. It must be pointed out that in this connection the surface of wheat is not merely the outer surface of the berry but, since water can penetrate wheat, it includes also the enormously greater *internal surfaces*. Heat is liberated progressively as water penetrates the wheat berry and so comes in contact with the internal surfaces.

This combination between wheat (and other colloidal materials) and water also results in a contraction in the total volume of the mixture. That is,  $x$  cc. of wheat plus  $y$  cc. of water when mixed will occupy a volume slightly less than  $x$  plus  $y$  cc., and this shrinkage will increase progressively until the water has become uniformly distributed throughout the wheat.

Since it is easier to detect and measure small changes in volume than small differences in temperature or in heat production it was thought that the volume-change method might afford a simple means of studying the rate of penetration of wheat by water. If this were the case it should be possible by the same simple means to correlate rate of movement of water in wheat with the original moisture content of the wheat, with type of wheat, with size of berry, possibly with protein content, and even with quality of gluten.

As will be shown later the method has not proved successful owing to certain peculiarities in the structure of the wheat berry, but its study has yielded results of considerable interest and value.

The method of work was extremely simple; the apparatus used is shown in Figure 1. Glass bottles of about 200 cc. capacity were fitted with rubber stoppers carrying lengths of about 30 cm. of fine capillary glass tube. The lower end of the capillary was flush with the bottom of the rubber stopper. A scale, graduated in cm. and mm., was attached to each capillary tube. The bore of each capillary was calibrated with respect to volume in the usual way by means of a mercury column. The capillaries were found to be very uniform in

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bore so that the average internal volume per cm. of length could be used without introducing any appreciable error.

Dimensions of three capillaries used were as follows:

	A	B	C
Length of mercury column, <i>i.e.</i> , of tube..	27.5 cm.	28.3 cm.	27.1 cm.
Weight of mercury column.....	6.2580 g.	6.3935 g.	6.1900 g.
Density of mercury at 20° C. ....	13.546	13.546	13.546
Volume of mercury column—weight divided by density.....	0.4620 cc.	0.4720 cc.	0.4569 cc.
Volume of mercury column divided by length in cm.—vol. per cm. length.....	0.0168 cc.	0.0167 cc.	0.0169 cc.

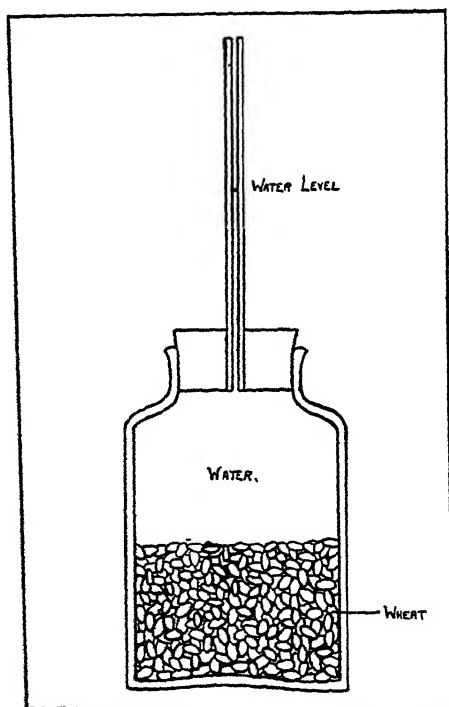


Figure 1

All experiments were carried out in a thermostat at 25° C. The volume changes may be expressed as rise (plus) or fall (minus) in cm. of solution above or below the original height in the capillary. These distances may be converted into volumes (cc.) by multiplying by 0.0168, and these volumes into percentage expansion or contraction of wheat by multiplying by 100 and dividing by the volume of 50 g. of wheat (50 g. of No. 1 Manitoba wheat used occupied a volume measured by displacement of 35.3 cc.). The wheats, the bottles, and

the water or solutions used were kept in the thermostat for at least 12 hours (usually overnight) before use, so that all the materials were at the same temperature, 25° C., before being mixed. Blank experiments were first carried out with water only in the bottles to ascertain whether slight movements of the rubber stoppers were likely to occur during the course of an experiment; such slight movements would cause the water to rise or fall in the capillary tube and so would vitiate the results. Such movements would necessitate the use of ground-glass stoppers. No movements occurred in such blank experiments, indicating that the technique was satisfactory.

The procedure in each experiment was as follows: 50 g. (to the nearest grain of wheat) of hand-picked wheat were placed in a bottle and kept in the thermostat overnight. The wheat was then well covered with distilled water (or solution) which had also been kept in a closed flask in the thermostat overnight, after having been freshly boiled and cooled to drive off dissolved air. The rubber stopper (with capillary tube) was placed in position, the capillary connected by rubber pressure tubing to a filter pump, and the pump turned on for about five minutes, during which time the bottle was occasionally shaken. This procedure removed all air bubbles entangled in the wheat, especially in beards and creases. When no more air bubbles were observable, the pump was disconnected, the bottle filled with water, and the rubber stopper pressed into the bottle sufficiently far for the water to rise to a convenient point near the top of the capillary tube. In every case the first measurement of the height of the water in the capillary was made ten minutes after the first addition of water to the wheat.

In the first experiments with No. 1 N. Manitoba wheat and water an increase in volume occurred during the first 1½ hours, as was indicated by the water rising in the capillary tube, and this was followed by a progressive contraction as shown by the water falling in the capillary. After about twenty-four hours, however, bubbles of gas accumulated in the bottle, the amount of gas increasing with time. In some experiments with English wheat gassing commenced after twelve hours. This gas could only be due to the liberation of absorbed air from the interior of the wheat, to incipient germination of the wheat, or to bacterial or mould action. It is stated in the literature that nitrobenzene solution will inhibit germination and mercuric chloride is a well known bactericide. Two replicate series of four experiments each were carried out with No. 1 N. Manitoba and with English wheat using respectively water, a 0.1% solution of nitrobenzene, a 0.1% solution of mercuric chloride, and a mixed solution of nitrobenzene and mercuric chloride of the same strength. In the

course of a week gas bubbles were produced only in the bottles containing wheat and water, a result which suggests strongly that the gas production observed was due to germination or to bacterial action or to both. In all subsequent experiments a solution of mixed nitrobenzene and mercuric chloride, of a concentration 0.1% in respect of each, was used instead of water alone.

The first wheat studied was No. 1 N. Manitoba of 13.39% moisture content. Duplicate experiments were carried out with water alone and with nitrobenzene-mercuric chloride solution. The results given in Table I show the close concordance between duplicate determinations. A further sample of the same wheat was moistened to 16.08% moisture content and allowed to lie 48 hours before being examined.

TABLE I

VOLUME CHANGES OF MIXTURES OF 50 GRAMS NO. 1 N. MANITOBA WHEAT AND WATER (OR SOLUTION)

(Volume changes are expressed as cm. rise (plus) or fall (minus) of solution in the capillary tube.)

Time after 1st reading	Moisture content 13.39%			
	Water alone		Solution	
Hours	cm.	cm.	cm.	cm.
0	0	0	0	0
$\frac{1}{2}$	—	—	—	—
$\frac{3}{4}$	—	—	—	—
1	+ 3.35	+ 3.3	+ 2.0	+ 2.2
$1\frac{1}{2}$	+ 3.9	+ 3.55	+ 2.45	+ 2.7
$1\frac{3}{4}$	—	—	—	—
2	+ 2.25	+ 2.1	—	—
$2\frac{1}{2}$	- 0.10	- 0.10	—	—
$2\frac{3}{4}$	—	—	—	—
3	- 2.4	- 2.55	—	—
$3\frac{1}{2}$	—	—	- 8.5	- 8.1
4	- 6.7	- 6.4	—	—
$4\frac{1}{2}$	—	—	—	—
$4\frac{3}{4}$	—	—	- 10.8	- 10.5
$5\frac{1}{2}$	- 10.1	- 9.15	—	—
$5\frac{3}{4}$	—	—	- 12.9	- 12.5
6	—	—	—	—
$6\frac{1}{2}$	- 11.75	- 11.25	—	—
$6\frac{3}{4}$	—	—	- 14.9	- 14.6
7	—	—	—	—
$7\frac{1}{4}$	—	—	—	—
$7\frac{3}{4}$	- 12.75	- 12.35	—	—
12	—	—	—	—
$22\frac{3}{4}$	—	—	—	—
$23\frac{1}{2}$	—	—	—	—
$23\frac{3}{4}$	—	—	—	—
$29\frac{1}{2}$	—	—	- 33.7	- 33.6
$31\frac{1}{2}$	—	—	- 36.0	- 35.9
47	—	—	—	—
$53\frac{1}{2}$	—	—	- 38.5	- 38.7
$71\frac{1}{2}$	—	—	—	—
122	—	—	- 38.9	- 38.9

Another sample was partially dried in an air oven at 120° C. to 10.87% moisture content. The detailed results are given in Figure 2.

Figure 2 is illuminating. Curves A and B may be regarded as the normal type of curve obtained with colloidal materials in general. The initial rise to a maximum is due to an expansion of the system caused by the heat of wetting, or rather it represents a balance between the expansion due to heat production and the contraction which takes place progressively from the start. The heat produced is slowly dissipated and after some time only the progressive contraction is observed. If the initial moisture content of the wheat is increased,

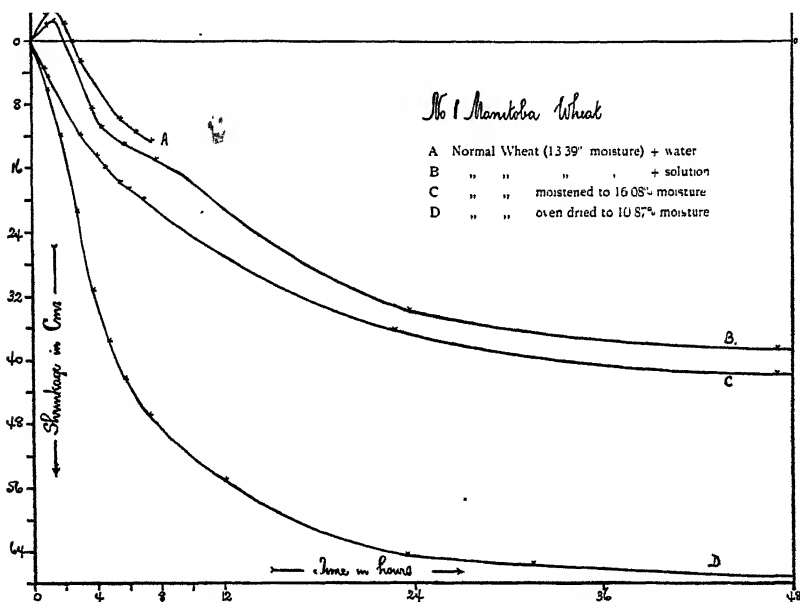


Figure 2

both the initial expansion and subsequent contraction should be reduced. It will be seen from Curve C (Fig. 2) that the initial expansion did not occur when the initial moisture content of the wheat was raised to 16.08% although it was very marked at 13.39%. The contractions were closely similar at both moisture contents. At the same time some heat must have been produced and the disappearance of the initial expansion can only be explained by the production of fine cracks and fissures, or by the increase in size of pre-existing cracks, during the preliminary moistening of the wheat. It is obvious that if such cracks were present or produced, the more or less rapid filling of these would produce a relatively sudden and greatly increased initial



contraction of the volume of the mixture, which would mask completely the initial expansion due to heat production.

This idea is strengthened by Curve D (Fig. 2). During the rapid drying of wheat at 120° C. one would expect fine cracks to develop as they do in timber during too rapid kiln drying. The partially dried wheat showed no initial expansion due to heat production but the contraction was very marked and particularly rapid in the first few hours. The total contraction in 48 hours equalled 1.12 cc. or approximately 3.2% of the total volume of the wheat. The highest total apparent contraction so far recorded with wheats was with a sample of Australian, oven dried to 11.17% moisture content: this showed an apparent contraction in five days of 5.2% of its volume. These values seem altogether too high if the observed contractions are real ones due to imbibition of water by the wheat endosperm. Thus, the phenomenon is more striking with gelatin than with wheat, yet with bone-dry gelatin the total contraction is only 4.0% (approximately) and with a 70% gelatin gel is about 1.7% of the volume of the gel.

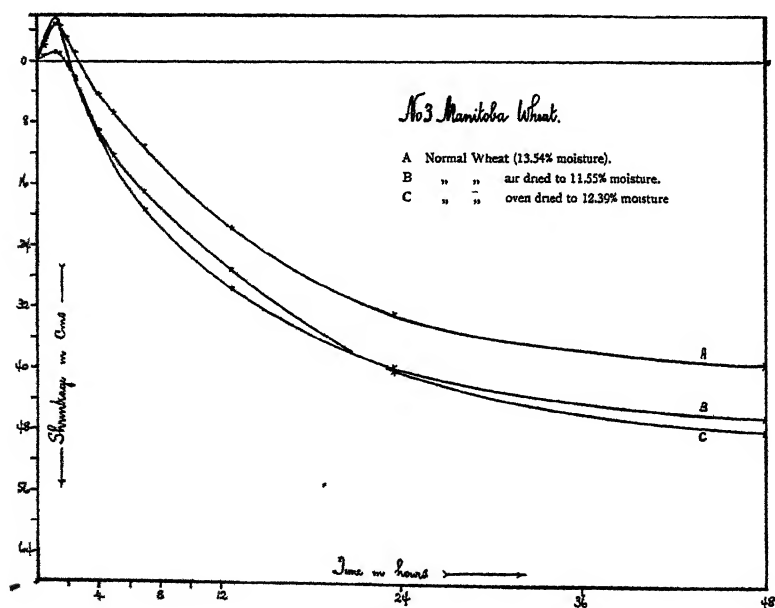


Figure 3

In Figure 3 are given the results obtained with a sample of No. 3 N. Manitoba wheat with an original moisture content of 13.54%. The normal curve (A) is similar in type to that obtained with No. 1 N. Manitoba and shows an initial expansion followed by progressive

contraction. Also, the total contraction in 48 hours was the same in both samples, 38.6 and 39.0 cm. respectively. After removing 2% of moisture by careful air drying at ordinary temperature the initial expansion showed little change while the total contraction in 48 hours was increased from 39.0 to 45.9 cm. On the other hand, removing only half the amount of water by the more drastic process of oven drying almost removed the initial expansion and increased the total contraction (in 48 hours) from 39.0 to 47.6 cm.

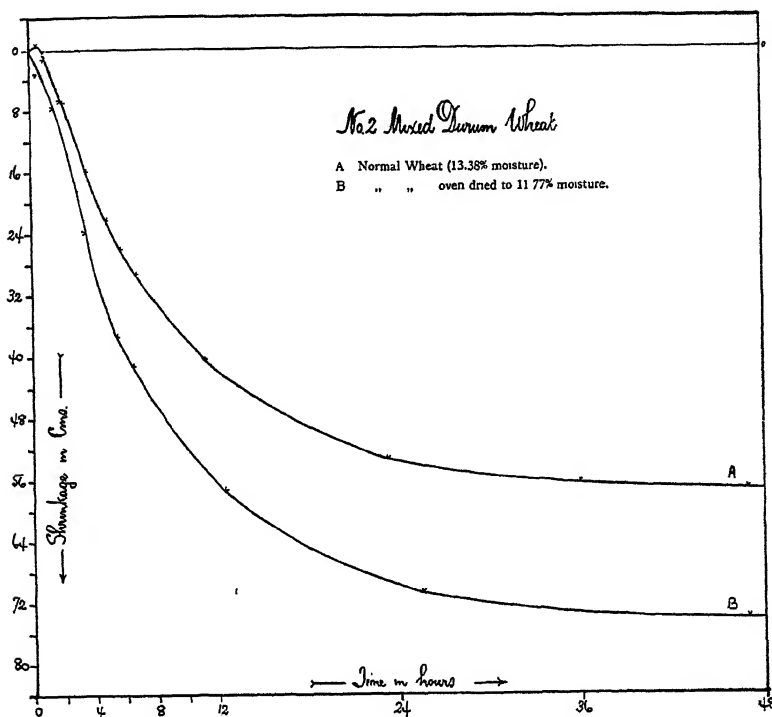


Figure 4

Some results with durum wheat are shown in Figure 4. The normal curve A shows only a slight initial expansion. Removing 1.5% moisture by oven drying obliterated the initial expansion and increased the total contraction from 57.4 to 74.0 cm.

Experiments with Red Standard and Yeoman English wheats are summarised in Figure 5. The normal Red Standard, with an initial moisture content of 15.46% (Curve A), showed a greater and a more prolonged expansion (heat evolution) than any of the other wheats examined: the maximum expansion was 10.3 cm. and no actual con-

traction from the original volume was observed for  $5\frac{1}{2}$  hours. Removal of only 0.8% of the moisture by air drying at ordinary temperature reduced the initial expansion and increased markedly the total contraction. The removal of  $3\frac{1}{4}$ % of moisture by oven drying obliterated the initial expansion and increased greatly both the total and the rate of contraction. The normal curve for Yeoman wheat (moisture content 14.87%) is also shown (Curve D).

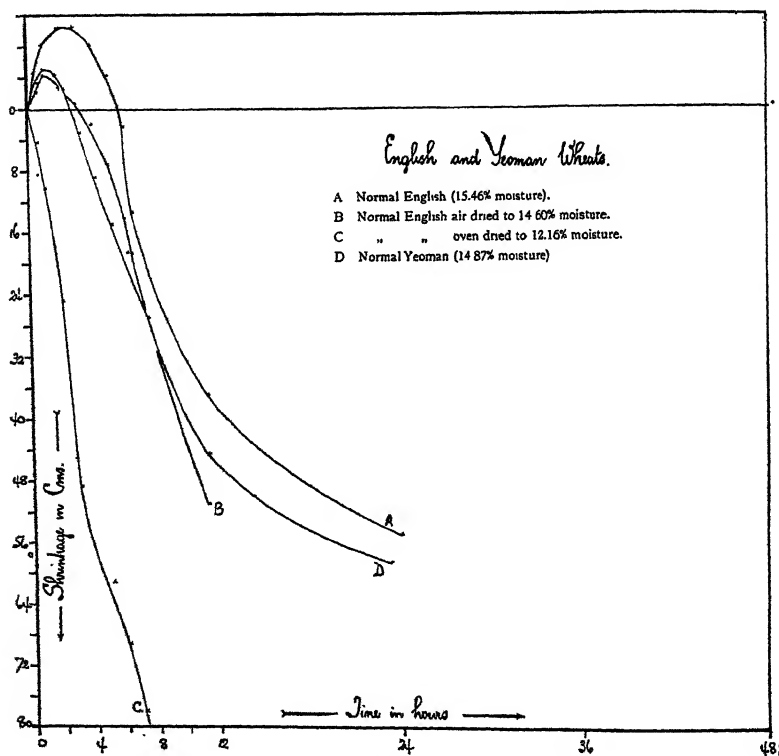


Figure 5

Some results for Plate wheat are given in Figure 6.

A more detailed set of results was obtained with a sample of Australian wheat and these are summarised in Figure 7. The natural wheat, with a moisture content of 15.40%, gave the normal type of curve with initial expansion followed by progressive contraction. Removal of 2.1% of moisture by air drying reduced the initial expansion and increased the total contraction by about 14 cm. Removal of a further 2.1% of moisture had no further effect on the initial expansion but increased the total contraction by a further 24 cm. Oven drying

to the same extents completely removed the initial expansion and increased the total contractions by 51 and 77 cm. respectively (compared with 14 and 38 cm. respectively for the corresponding air-dried samples).

The air-dried sample (moisture content 11.17%) was re-moistened to 15.40% moisture by standing over water in a closed vessel. This procedure removed entirely the initial expansion and slightly increased (by about 2 cm.) the total contraction. In other words, adding

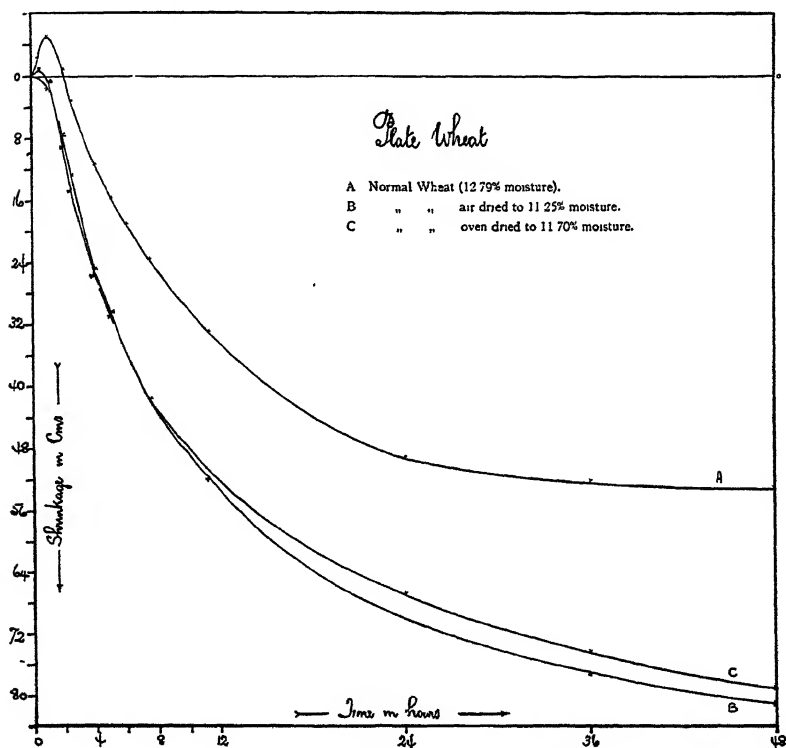


Figure 6

moisture carefully in the form of vapour to the air-dried sample did not cause the wheat to return to its original mechanical condition: Curve F is very different from Curve A.

These apparent anomalies are probably due to the presence of permanent strains set up in the wheat berry as a result of the stresses imposed by the desiccation of the wheat during ripening. These strains would vary with subsequent addition or subtraction of water; actual distortion or even cracking (microscopic) of the wheat would

occur, and the more rapid the removal of water, as in oven drying, the greater the distortion and cracking. In other words, the mechanical and physical properties of a sample of wheat depend to some extent on its "moisture history." Thus it has been shown by Sharp (1927)

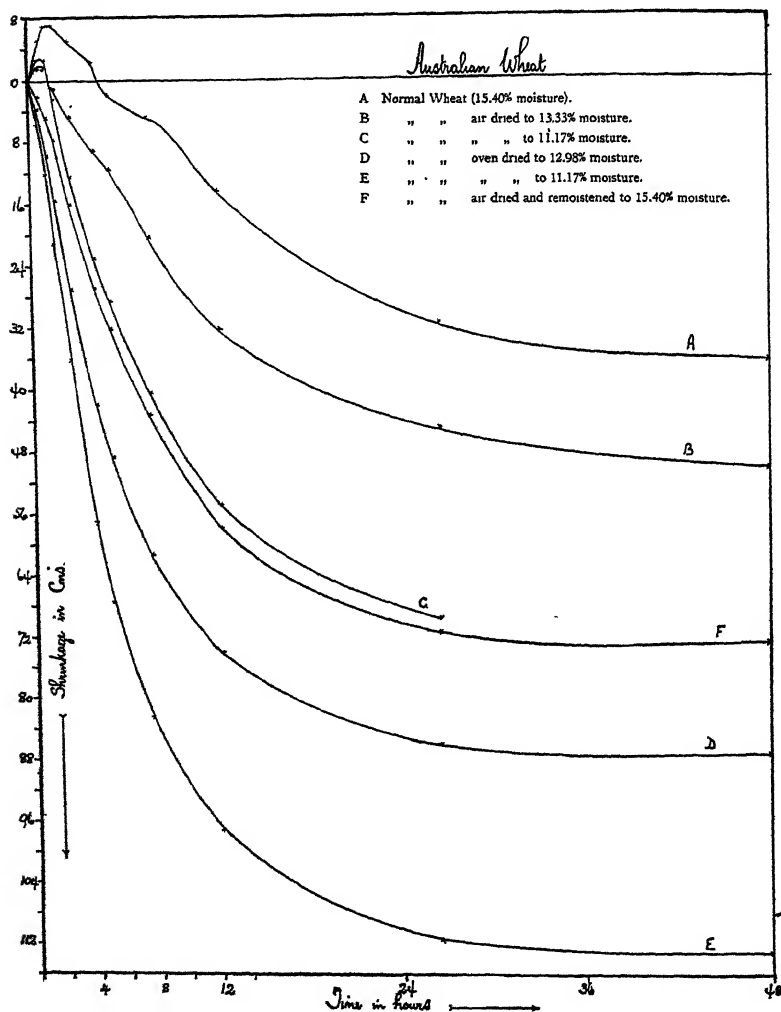


Figure 7

that the apparent density of wheat depends on its "moisture history." If moisture is added to wheat its density is decreased, but if the moistened wheat is carefully re-dried to its original moisture content the original density is not regained; the wheat remains definitely of slightly

lower density. This again falls into line with the earlier work of Thomas (1917), who showed that the bushel weight decreases with increasing moisture content; that is, an increase of 1% moisture means a decrease of approximately  $\frac{3}{4}$  lb. in bushel weight. If the moistened wheat is carefully re-dried to its original moisture content, the original bushel weight is not regained but remains definitely lower. Moreover, this permanent decrease in bushel weight is proportional to the extent to which the moisture content was raised prior to re-drying. Thomas's figures for one sample of spring wheat are given in Table II.

TABLE II  
EFFECT OF MOISTURE CONTENT ON THE BUSHEL WEIGHT OF A SAMPLE OF WHEAT  
(L. M. THOMAS)

Moisture content	Bushel weight	Moisture content after re-drying	Bushel weight after re-drying
%	lbs.	%	lbs.
11.4	63.0	—	—
13.2	61.0	11.1	62.0
15.2	60.0	11.3	61.5
18.8	56.0	11.0	61.0
22.2	53.0	11.0	60.5
25.2	52.0	11.4	60.0

These results of Thomas have been broadly confirmed in the present writers' laboratory, although the problem appears to be even more complicated than is suggested by Thomas's figures. After wheat is moistened, the bushel weight falls but subsequently rises progressively on standing for several days until a maximum value is obtained; this maximum value is always less, however, than the original value obtained before moistening. This holds up to about 20% moisture content, above which, for various reasons, bushel weight determinations tend to be erratic.

The phenomenon is not without practical significance. It may be a factor in the difference sometimes observed in the bushel weight of a cargo of foreign wheat as recorded in the countries of import and of export respectively, even when difference in moisture content is taken into consideration.

One sample of mixed Nos. 1, 2, and 3 N. Manitoba wheat was investigated at two temperatures, *viz.*, 25° C. (77° F.) and 37.5° C. (99.5° F.). The results are summarised in Figure 8. The rate of shrinkage was at first greater with the warmer sample but the rate diminished earlier with this sample so that at 28 hours the total shrinkage was the same for both. After about 15 to 20 hours the rate of shrinkage became the greater with the colder sample and remained so up to six days, when the experiment was discontinued.

This effect of temperature on rate of apparent absorption of water by wheat is roughly in line with what is known about gelatin and other colloids: the higher the temperature the less water is bound by the colloid and hence the total shrinkage is less. With gelatin, however, the initial rate of shrinkage is also less at higher temperatures, while with wheat the reverse appears to be the case. This reversal is presumably due to greater distortion of wheat at higher temperatures. The total shrinkage must depend on two factors—the filling up of cracks and the real imbibition of water by wheat endosperm. The relative magnitudes of these two factors cannot be assessed, nor is it likely that the ratio of the two factors will remain constant throughout the course of a single experiment.

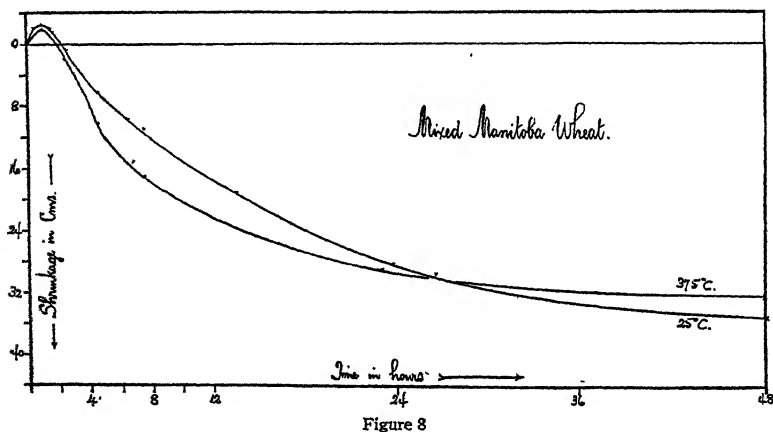


Figure 8

This experiment throws an interesting light on the mechanism of hot conditioning. At the higher temperature wheat will take up moisture more quickly, but this take-up is largely mechanical since actually less water is bound to the endosperm than is the case at lower temperatures. After the hot conditioned wheat has been cooled and is lying in the bin a further proportion of the water inside the wheat becomes bound by the endosperm without further movement.<sup>1</sup> It is possible that this may be an essential part of the tempering process that takes place in wheat when lying after hot conditioning.

As a practical method of investigation the volume-change method has proved somewhat disappointing. The results recorded do, however, throw some light on the mechanical structure of the wheat berry and on the mechanism of wheat conditioning. They also agree with

<sup>1</sup> This, of course, follows from purely thermo-dynamic considerations. Since heat is evolved by absorption of moisture by a colloid, the extent of the absorption must be less the higher the temperature in accordance with the well known Le Chatelier principle. For an interesting discussion of the general problem of the thermo-dynamics of water absorption, see Shorter (1924).

those discussed in an earlier paper (Fisher and Jones, 1939) in showing that very little moisture movement takes place after three days; with the mixed Manitoba sample the total shrinkage during the second three days was less than 3% of that of the first three days, and considerably less than half that of the third day.

### Summary

When a colloidal material, such as wool, cotton, gelatin, or wheat, and water are mixed and allowed to stand at constant temperature a progressive shrinkage of the mixture occurs. Thus, if  $x$  cc. of wheat and  $y$  cc. of water are mixed the volume of the mixture will be slightly less than  $x$  plus  $y$  cc. and the shrinkage will increase progressively until the water is uniformly distributed throughout the wheat.

An attempt has been made, by a simple but accurate method, to measure these volume changes over periods of days in mixtures of wheat and water. The rates of such volume changes should be related to the rates of movement of water throughout the wheats, to the original moisture content of the wheat, and to other factors. The results obtained, although of considerable interest, are anomalous and can only be interpreted as indicating the presence of fine cracks or fissures in the wheat, or the production of fine cracks or an increase in size of pre-existing cracks during the moistening. Such cracks make it impossible to measure the real shrinkage that undoubtedly occurs.

It is suggested that the cracks may be caused by the presence of permanent strains set up in the wheat berry as a result of the stresses imposed by the desiccation of the grain during ripening. These strains would vary with subsequent addition or subtraction of water, and actual distortion and microscopic cracking of the wheat would result. Such a mechanical condition of wheat would explain various phenomena such as the facts that wheat density and bushel weight are affected by the previous "moisture history" of the wheat.

The effect of temperature is characteristic: a higher temperature appears to hasten materially the movement of water in the earlier stages, *e.g.*, up to 12 hours or so, but to decrease the rate subsequently. The total shrinkage is also less at the higher temperature. This is roughly in line with what is known of other colloids and appears to throw some light on the mechanism of wheat conditioning.

### Acknowledgment

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**SOME REMARKS ON THE VARYING INFLUENCE OF  
COMPRESSED YEASTS OF DIFFERENT INDUSTRIAL  
ORIGIN ON THE GAS RETENTION OF DOUGH,  
AS RECORDED BY A NEW INSTRUMENT,  
THE CHEFARO BALANCE<sup>1</sup>**

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Cereal chemists, wishing to eliminate the personal factor of the baker's skill in baking experiments, have devoted much time to elaborate experimental methods, whereby one can predict what sort of loaf may be produced with definite ingredients, and it is generally recognized that it is necessary in this endeavor to study a large number of different properties. This, however, requires much time, and therefore during recent years efforts have been made to construct a recording apparatus to produce curves that constitute a basis for evaluating the more important properties of the dough.

Mueller (1937) gives a historical survey of some mechanical methods for the determination of flour and dough properties and mentions among others Boland's "aleurometer" for the determination of gluten quality, constructed in 1880; Liebermann's apparatus for the same purpose; Kosutany's method for the determination of the physical properties of dough, published in 1907, which emphasized the fact that it is much better to study the whole dough rather than the gluten alone; Hankoczy's apparatus, which gives numbers for the quality, the extensibility, and the elasticity of the gluten; Chopin's "extensimeter," constructed independently in 1921, based on the extensibility of a dough; Bühler's "comparator," constructed in 1924 and Barbade's

<sup>1</sup> Paper read before Section VIII on Agricultural and Industrial Microbiology of the Third International Congress for Microbiology, New York City, September 2-9, 1939.

"aleurograph" (1928), both based on the same principle as Chopin's "extensimeter." All these contrivances determine gluten or dough qualities shortly after mixing, and do not permit measurements of dough properties during the rest of the bread-making process.

Mueller (1937) further describes mechanical devices based on the force required for mixing the dough; he mentions Hogarth, Hankoczy, Deutschrenner, Bailey, and Brabender's farinograph. Although no effort is made here to present a complete review, mention may further be made of Swanson and Working's recording dough mixer; a Belgian apparatus called Varmi's "patograph," based on the same principle as Brabender's farinograph; Chopin's recording mixing apparatus, which delivers mechanically the dough disks required for his "alveograph," which is an improved form of his "extensimeter."

Gas production during the fermentation of dough is an extremely important factor and some methods for its determination are described by Elion (1933). Recording apparatuses for gas production are, for example, Brabender's "fermentograph" and some recent Belgian contrivances, such as Varmi's "volumetrograph," which records the total gas production and the volume obtained by the fermenting dough in a cylinder, and Varmi's "panigazograph," which measures the gas produced during a baking process.

For the purpose of determining gas retention by the dough during fermentation and the factors influencing it, no recording apparatus has been available which would enable one to follow the gas retention *during the whole period of the fermentation* instead of only during some short periods thereof. It is not sufficient merely to know the quality at the beginning of the fermentation, at some definite moment during the fermentation, or after the fermentation (finished loaf). The influence of diastatic and proteolytic enzymes is so important that it is desirable to record during the whole period of fermentation.

This requirement has been met by the Chefaro balance, developed and patented by the Chemische Fabriek Rotterdam, Rotterdam, Holland. Before discussing the experiments made with this apparatus, a description will be given.

### The Recording Chefaro Balance

The Chefaro balance (Fig. 1) is an analytical precision balance, by which the quantities of gas retained and produced by the fermenting dough can be accurately weighed and automatically recorded. In a special gas-jar (Fig. 2), which is open at the bottom, a dough ball is placed on a small scale and the gas-jar is attached to one beam of a balance, after being immersed in a water bath which is electrically heated and kept automatically at the desired temperature. A special

construction allows the liquid of the bath to rise into the gas-jar until a definite point under the scale is reached. The other beam of the balance bears a counter-weight which can be moved and has at its end a special pen. The recording drum is rotated by a clock and moved on and back again by an electric motor; the pen writes 30 points per minute in an unbroken line on the graphs. This system eliminates any friction during recording.

Each apparatus consists of two complete balances, the two special gas-jars being immersed in the same water bath.

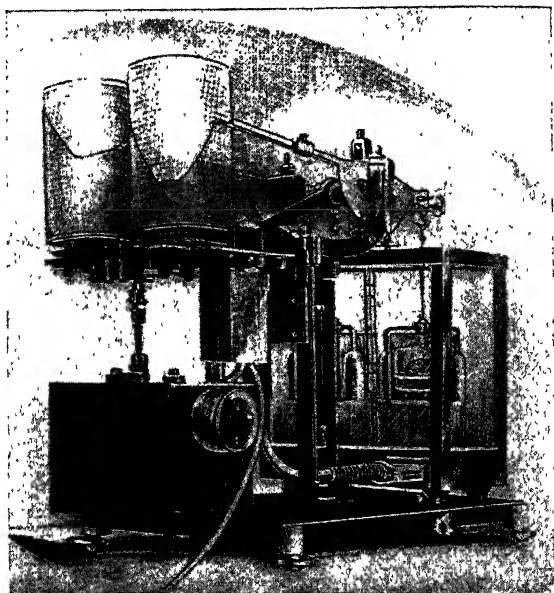


Fig. 1. The Chefaro balance.

The gas produced by the fermenting dough displaces liquid from the gas-jar, which therefore becomes lighter and rises in the water bath; the writing pen at the end of the balance beam descends accordingly and the gas production is recorded. It is necessary of course that the gas produced be insoluble in the liquid of the bath, for which purpose the manufacturers supply a liquid to be mixed with the water in the water bath.

If gas retention is to be recorded, ordinary water can be used in the water bath and on the lower compartment of the small scale in the gas-jar a solid material is placed, which absorbs the carbonic acid escaping from the dough, so that only the increase of the dough volume, *i.e.* gas retention by the dough during the whole fermentation period, is recorded.

The manufacturers give full details as to the manufacture of the small doughs required for the experiments. Water absorption of each flour is determined with a centrifuge and the percentage of water found in this way is used in the making of the small dough. Each flour therefore obtains its own quantity of water and for the purpose of obtaining comparable results, the doughs placed on the scales are weighed so as to contain each the same quantity of flour, three grams of flour being used for the determination of gas retention. If one would use doughs of the same total weight, the stronger flour would be at a disadvantage as compared with weaker flour, since a dough made from the latter with the same weight of dough would contain less water and more flour

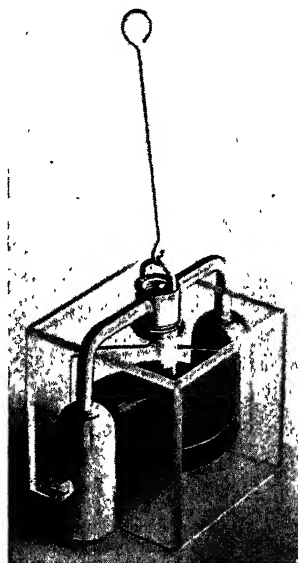


Fig. 2. The gas-jar.

and yeast than a dough made from a stronger flour. To compensate for the small volume of the dough, extra yeast and sugar are employed. When determining total gas production, even smaller doughs can be used, containing one gram of flour only.

### Significance of the Curves

The curves in Figure 3 show the following factors: (a) the gas-retaining capacity or the highest gas-pressure the dough can support, expressed in cubic centimeters expansion of the dough ball, (b) time required until the full development of the dough, and (c) the stability of the dough after reaching full development. Flour sample *I* has a slow fermentation; the dough can tolerate only a very slight expansion

and the stability is also inferior. Sample *II* ferments much more quickly; the gas-retaining capacity and the stability are much greater. Sample *III* has a somewhat slower fermentation, but the gas retention and the stability of the dough are the best of the three.

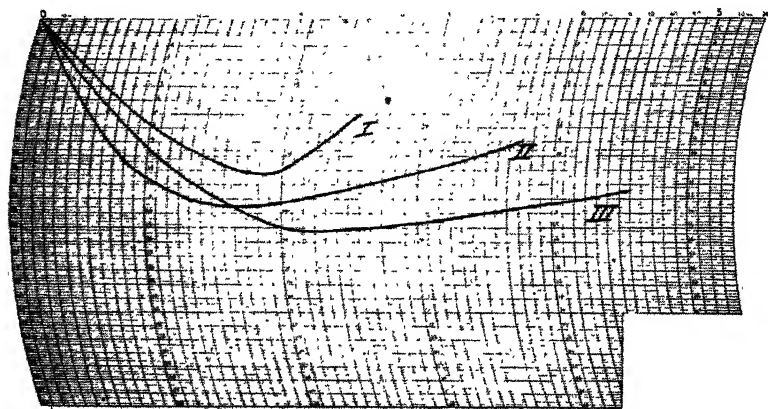


Fig. 3. Curves showing different dough properties.

### Influence of Flour Improvers

The Chefaro balance is especially suitable for showing the influence of different flour improvers on the baking quality of flour samples, and for indicating the quantity of improver which gives the best results, since gas retention during the whole period of the fermentation is recorded. Improvers may change the curves considerably, and overtreatment may easily be demonstrated by means of the gas-retention curves.

### Influence of Different Yeasts on the Gas Retention of Dough

As the Chefaro balance records gas production and gas retention of dough during the whole period of fermentation, it is especially adaptable for a study of the influence of compressed yeasts of different industrial origin on the gas retention.

It may be expected that gas retention depends on gas production, since lack of gas production will prevent a dough with good gluten qualities from reaching the maximum volume consistent with the gas-retention capacity of the dough. For the purpose of the present experiments it would therefore be desirable to deal with different yeasts which give approximately the same gas production in doughs made from a definite flour sample, and this has indeed been possible.

As a suitable flour for this purpose a Netherlands inland flour was chosen, which had a water absorption capacity of 58.3%. According

to the instructions of the manufacturers of the Chefaro balance, the doughs were made of 5 g. of flour, with addition of 4% yeast, 4% glucose, 2% NaCl, and the necessary water according to an absorption of 58.3%. From one dough were weighed: (1) a dough containing 3 g. of flour for the determination of gas retention and (2) a dough containing 1 g. of flour for the determination of total gas production. Both determinations were made at the same time in one water bath, which was kept at a temperature of 35° C. In this way a large number of experiments have been made with the same flour and different compressed yeasts, originating from different European countries and kindly placed at our disposal by a number of yeast manufacturers.

It is desirable in these experiments to compare yeast samples of the same freshness, and to perform the tests under the same conditions. Under these circumstances duplicate determinations give curves which correspond very well. This is illustrated by the curves of Figure 4, which have been taken by way of example. Curves *I* and *II* are duplicate gas-production curves and *III* and *IV* duplicate gas-retention curves.

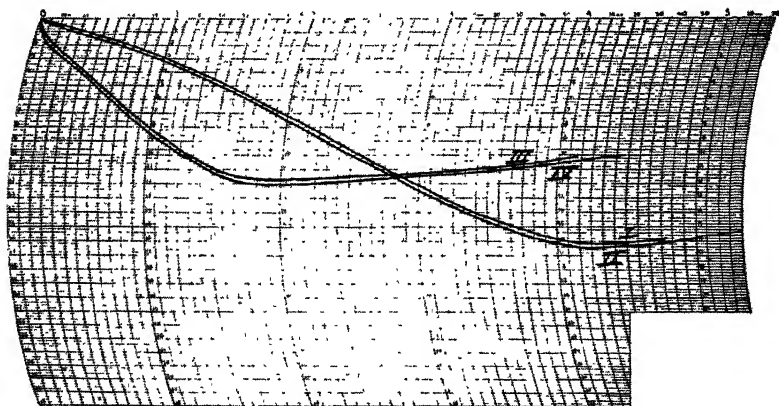


Fig. 4. Agreement of duplicate tests for gas production and gas retention.

Figure 5 gives by way of example the results obtained with four different yeasts. The four gas-production curves are indicated with *I* and the four gas-retention curves with *II*.

It may be concluded that the gas-production curves are similar in character, although the absolute value for gas production in one case after 3 hours differs from the others. The gas-retention curves, however, show some remarkable differences. In the first place two curves only (broken lines) have a regular course comparable to curves *III* and *IV* of Figure 4, but the two others (full lines) show after about 50

minutes an interruption of the regular course. This indicates a sudden escape of gas from the dough and a corresponding decrease of its volume, after which the further gas production causes a new increase of the dough volume. At the moment of the sudden escape of gas, the dough was obviously incapable of retaining all the gas, probably because there was at that moment no good proportion between gas production and gas retention capacity and as a consequence the maximum volume reached by the dough is smaller than in the case where a sudden escape of gas did not occur. Furthermore the gas-retention curves of the different yeasts during the second and third hour show relatively great differences in gas volume retained by the dough, although the gas production curves do not show such important differences in the corresponding periods.

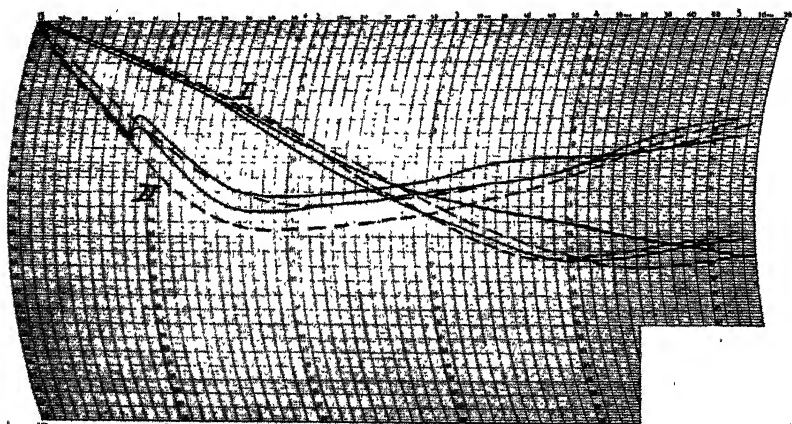


Fig. 5. Variations in gas production and gas retention with different yeasts.

This indicates that the four yeasts have a different influence on the gas-retention capacity of the dough made from the same flour under the conditions of these experiments, a difference which is not caused by differences in gas production.

According to the gas-retention curves obtained, the yeasts can be classed into two groups: those giving a sudden escape of gas (full lines) and those not giving such an escape of gas (broken lines). It is very interesting to note that the yeasts which caused a sudden escape of gas were yeasts made from molasses mashes, while the yeasts which did not cause such an escape of gas were made from grain mashes. We have studied a larger number of yeasts, made of grain mashes as well as of molasses mashes, and all the yeasts have given the same kind of gas-retention curves; *i.e.*, all the molasses yeasts caused a sudden escape of gas from the dough after approximately 50 minutes, while

all the grain yeasts did not cause any sudden escape of carbonic acid from the dough.

This particular difference between the two kinds of yeasts seems to be somewhat related to flour quality, as shown by some preliminary experiments with other flours. It may not be as easily demonstrable with all flours, but the time available for submitting the abstract of this paper to the Congress has made it impossible to investigate this question. The matter will be studied at a later date. For the same reason it has been impossible to study thoroughly the question as to why yeasts made from a molasses mash have a different influence from that of the grain mash yeasts on the gas retention of dough made from the flour represented in Figure 5. We intend to study this question further, also, but some observations may be given here.

Gluten quality has a great influence on gas retention of dough. Flour contains proteolytic enzymes, which can attack the flour proteins and may therefore have an unfavorable influence on gluten quality. The possibility arises that the varying influence of different yeasts on gas retention has something to do with proteolytic activity in the dough.

It is generally admitted, however, that living yeast persistently retains its proteinase. According to R. Willstätter and W. Grassmann (1926), living yeast cells which are not damaged do not secrete proteinases. According to W. Grassmann and H. Dyckerhoff (1928) and W. Grassmann, O. v. Schoenebeck and H. Eibeler (1931) yeast, even when autolysed in the presence of cell poisons, secretes only very insignificant quantities of proteinases during the first hours; only after a duration of the autolysis for about 15 hours does the secretion of proteinases begin.

As far as the author knows, no experiments have been published which demonstrate the secretion of proteinases by living, undamaged yeast. Some papers dealing with yeast proteinase (R. Geoffroy and G. Labour, 1934; A. V. Blagoveschenski and M. P. Yurgenson, 1935) describe only experiments in which the yeast has been treated in the presence of cell poisons, such as toluene.

On the other hand, until some years ago it has been believed that flour contains only small quantities of proteinases. H. Jørgensen (1935, 1935a) developed a very interesting theory on the nature of the action of chemical flour improvers, such as potassium bromate. According to this theory  $\text{KBrO}_3$  and other flour improvers of its kind paralyze (more or less completely) the proteinases of the wheat flour. Under these circumstances the break-down of the proteins of the dough diminishes and, as a result, the gas-retaining capacity of the dough and the baking strength increase.



In a further paper relative to this subject, H. Jørgensen (1935b) concludes that the reduction in solubility of flour nitrogen that is produced by  $\text{KBrO}_3$  in flour-water suspensions is strongly increased by the addition of yeast. He explains this by assuming that the addition of yeast causes an increased activity of those proteinases in the suspension which can be inhibited by  $\text{KBrO}_3$ . He further concludes that this increased activity is not caused by proteinases originating from the yeast, but that the flour proteinases are activated by the presence of yeast. To prove the correctness of this conception, Jørgensen describes experiments with flour the proteinases of which have been destroyed or considerably reduced in activity by heating the flour for 12 hours at  $95^\circ \text{C}$ . Suspensions of such flour in water, to which yeast was added, gave no increase of the activity of the proteinases in question, while the proteins in the flour could still be attacked by other proteinases of the papain type.

These results of Jørgensen were obtained with dried yeast as well as with fresh yeast. The author states that in the case of dried yeast the activation of the proteinases in question may be caused by the fact that dried yeast secretes glutathione and that this glutathione activates the flour proteinases susceptible to  $\text{KBrO}_3$  in the same way as is done by Ambros and Harteneck's phytokinase (1928, 1929). Jørgensen demonstrates that glutathione may readily be extracted from dried yeast, the extract giving a positive reaction with sodium nitroprusside.

Although this theory on the effect of yeast on flour proteinases is very attractive, it does not explain how living yeast, which has not been damaged in any way, can activate the flour proteinases, since living yeast does not secrete glutathione, as may be demonstrated by the negative reaction with sodium nitroprusside.

In another paper H. Jørgensen (1936) shows further evidence of the existence of powerful but latent proteolytic enzymes in wheat flour, which can be stimulated by activators such as glutathione or yeast-water.

V. Carbonnelle (1938) determined the quantity of glutathione present in two yeasts made from grain mashes and in one yeast made from a molasses mash. He found that the quantity of glutathione in the molasses yeast was about 60% only of the quantity present in the grain yeasts. If indeed the glutathione of living yeast would exert in an unknown way any activating effect on the flour proteinases, *i.e.* a softening effect on the gluten, the curves found in our experiments for the gas retention of the doughs (Fig. 5) would be easily explained. The grain yeasts, containing more glutathione than the molasses yeasts, would according to Carbonnelle, weaken the gluten of the flour

more than would be done by the molasses yeasts. The fact that the doughs made with molasses yeasts (full lines in Fig. 5) showed a sudden escape of gas proves that at that moment the doughs were stiff and unable to retain the gas, whereas the doughs made from the grain yeasts became somewhat weaker by increased proteinase activity and consequently were able to retain the gas.

Although the results of our experiments would find an explanation in this way, it must be stated once more that further experimental evidence as to the influence of glutathione on flour proteinases in the case of living, undamaged yeast is needed.

Some remarks, however, may be made in this respect. W. Grassmann and H. Dyckerhoff (1928) discuss the activated and the non-activated forms of proteinases present in papain and in yeast. They draw attention to a distinct difference between these enzymes. The non-activated papain *requires* the presence of activating substances like hydrocyanic acid or Ambros and Harteneck's phytokinase for the hydrolysis of peptones, but for the splitting of proteins these activators are useful but *not necessary*. In the case of yeast proteinase, on the contrary, the addition of a biological activator or of an activator foreign to the cells is *necessary* for splitting proteins and *not necessary* for the hydrolysis of peptones.

A. K. Balls and W. S. Hale (1936, 1936a, 1938) demonstrated that flour proteinases are of the papain type.

In view of these observations, one might suppose that flour contains a biological activator which would enable the break-down of proteins by yeast proteinases. In experiments with heated flour Jørgensen (1935b) shows that the proteins in heated flour are attacked by papain but not by yeast, and he considers this fact as a support of his theory that yeast activates flour proteinases but does not secrete proteinases itself. As a result of heating the flour, its proteinases are destroyed or considerably reduced in activity, and it may be possible that this will also be the case with any biological activator of yeast proteinases, if present in the flour. Since papain, according to Grassmann and Dyckerhoff, should be able to attack proteins without addition of an activator, while yeast would *require* such an activator, the experiment of Jørgensen with heated flour-water suspensions and yeast is not quite convincing. In the case of the attack on the proteins of heated flour by yeast the addition of an activator, foreign to the yeast cell, might be required, and as heating the flour might have destroyed such an activator in the flour, if present, it would be necessary to add an activator to the experiment with heated flour and yeast. Perhaps glutathione might be able to serve as such an activator, since according

to Jørgensen glutathione alone is unable to attack the proteins of heated flour.

Jørgensen's theory on the nature of bromate action has found much approval among cereal chemists, but also some objections have been raised. Jørgensen (1938) discusses these publications and shows why some of the objections raised are wrong. He has not yet had the opportunity of discussing the objections raised by W. P. Ford and A. M. Maiden (1938). These authors added glutathione (GSH) to the dough and found with Brabender's farinograph that an addition of 0.005% GSH to the flour weakened the dough considerably in 10 minutes. Repeating the experiments with papain, they found that an addition of 0.03% of papain gave approximately the same curve in 10 minutes and they concluded that 0.03% papain would be equal to 0.005% GSH. These authors then left the doughs for two hours and found that after this time the papain doughs were much weaker than the GSH doughs. Ford and Maiden believe that *if* GSH really acts by stimulating the flour proteinases, the quantities of GSH which after 10 minutes gave the same results as did a definite quantity of papain should also duplicate the results with papain after two hours. Since this is not the case, Ford and Maiden conclude that GSH does not act on the consistency of the dough by stimulating the flour proteinases, but by a direct action on the proteins of the flour. They further state that if Jørgensen's theory of the action of glutathione is wrong, there is reason to doubt also his theory of the action of bromate.

Jørgensen (1938) states that according to a private communication received in 1937 from J. T. Flohil, Minneapolis, the latter made an observation similar to that of Ford and Maiden, but instead of glutathione he used wheat germ, which however contains glutathione, according to Sullivan, Howe and Schmalz (1936).

We do not have Flohil's communication. When considering the normal farinograph curves published by Ford and Maiden (1938) for a dough containing 0.005% glutathione or 0.03% papain, some doubt may arise as to whether these quantities are really to be considered as having the same effect on the dough during the first period after mixing, since the curves are not exactly the same. If this doubt would prove to be correct, one may not expect that these quantities will have the same effect after a period of two hours. Furthermore, in view of our observations mentioned above, glutathione would only be an *activator* of proteinases, while papain would be a complete *proteolytic enzyme* which does not require any activator for the break-down of flour proteins. If Ford and Maiden in one experiment add only 0.005% of a proteinase *activator* (GSH) and in the other experiment much more (0.03%) of a fresh *proteolytic enzyme*, one may hardly expect that

in the long run the small quantity of proteinase activator would be able to equal a much larger quantity of proteinase added (which may moreover itself contain an activator), even when the chosen quantities would give similar results on the farinograph during the first period after mixing.

A similar situation will perhaps apply to Flohil's experiments, since he used wheat germ, which contains glutathione.

The author hopes to be in a position to present further experimental evidence on this interesting subject in the future.

### Summary

The author describes a new apparatus, the recording Chefaro balance for the determination of gas retention and gas production during the whole period of fermentation of dough, which has been developed and patented by the Chemische Fabriek Rotterdam, Rotterdam, Holland. He furthermore describes his experiments with this apparatus, from which it appears that compressed yeasts of different industrial origin have a varying influence on the gas-retention capacity of dough. The author discusses some theories which might help to explain the differences found.

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## A COMPARISON OF THE ALLIS-CHALMERS AND THE BUHLER AUTOMATIC EXPERIMENTAL MILLS<sup>1</sup>

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(Received for publication May 22, 1939)

The production of flour by means of the experimental mill has always been a problem to cereal chemists because of the time-consuming nature of the procedure, the difficulty of training men to do the work, and the rather wide range of replication errors. There is no doubt that any automatic experimental mill capable of producing reasonably good flour would be very much welcomed by all cereal technologists who have to test wheat samples for baking quality.

Müller (1934) described an automatic mill made by the Brabender Company in which were used two pairs of stone-type grinders. The flow was simple and rapid. The mill was designed primarily to produce flour for the farinograph. Geddes and Aitken (1937) made a comprehensive comparison of flours produced on this mill and on the Allis-Chalmers mill and concluded that the flour from the Brabender mill is not suitable for use in differentiating hard Canadian wheats, although it gave fairly good results with softer types of wheat. There seems to be no record of any work on hard winter wheats with this mill.

In 1935 the Buhler Company of Uzwil, Switzerland, designed and built an automatic experimental mill which embodied many desirable

<sup>1</sup> Contribution No. 60 of the Department of Milling Industry, Kansas Agricultural Experiment Station, in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the state agricultural experiment stations of the hard winter wheat region.

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features. It has a three-break, three-reduction flow, which, although longer than the Brabender, is considerably shorter than the customary procedure with the Allis mill. A good description of this mill is given by Ziegler (1938).

A somewhat detailed discussion of the flow of the Buhler mill is given by Anderson (1938) in a comparison of commercially milled flours and flours milled by the Buhler mill, both from the same wheat mix. It was found that the flours from the Buhler mill were lower in diastatic activity, higher in ash, and about the same in protein content. The time required for the Buhler mill was materially less than for the Allis-Chalmers experimental mill. However, he concluded that the Buhler mill, although automatic in operation and amenable to very close adjustment, could not be operated to advantage by a novice, but required the supervision of a person having a good technical knowledge of milling.

The automatic features of this mill, and the nature of the flow employed in it, created so much interest that it was thought advisable to make some direct comparisons of the Buhler with the Allis-Chalmers mill, with which most cereal chemists are familiar. This should permit a useful appraisal of the performance of the Buhler mill. In this first comparison no attempt was made to compare flours with respect to baking quality, as was done in the work of Geddes and Aitken (1937). The chief purpose was to get an estimate of the degree of reproducibility of milling results.

Accordingly only one lot of wheat was used, and the flour samples were merely analyzed for moisture, ash, protein, and diastatic activity. The millings were made simultaneously on the Buhler and Allis-Chalmers mills, both of which were located in the same room. The work was performed by two millers experienced in the operation of both mills. The test was continued over a period of four days, the first two and the last two being consecutive. The millers alternated on the mills by days, so that each miller had two full days' work on each mill. No attempt was made to control the atmospheric conditions of the room during this test. The wheat milled during the first two days was tempered to 16% moisture in the usual manner. This moisture content, however, proved to be somewhat high for the Buhler mill, and for this reason the moisture was dropped to 15% for the last two days of milling, which was above the optimum for the Buhler but was a little too dry for the best operation of the Allis mill. Thus the wheat was closer to the optimum for the Allis mill on the first two days and closer to the optimum for the Buhler mill during the last two days. The analyses, however, show no significant differences that might be attributable to this procedure.

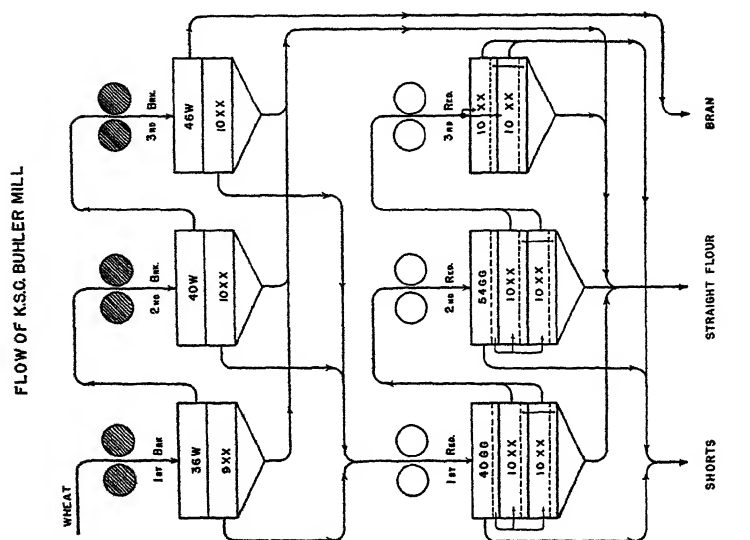


Fig. 2. Flow sheet of the Buhler mill.

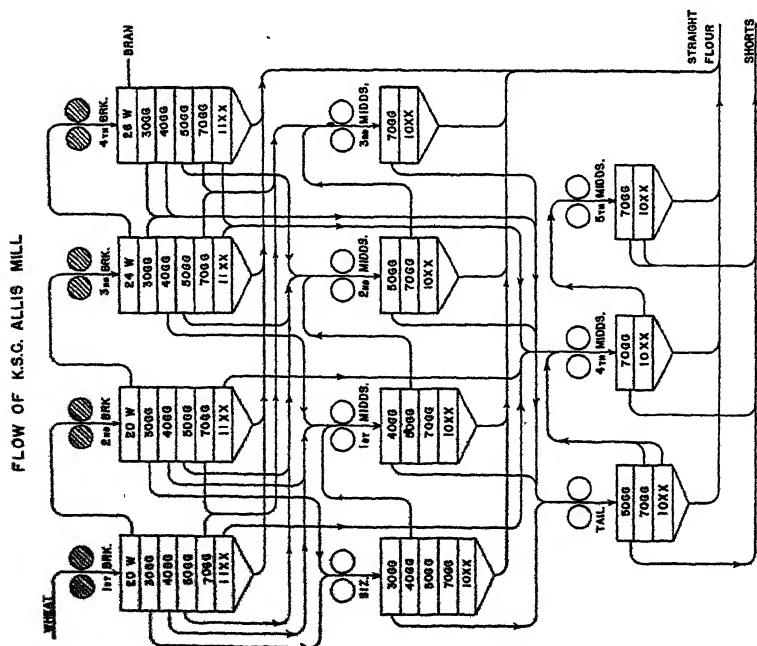


Fig. 1. Flow sheet of the Allis-Chalmers mill.

As it was desired that a full day's milling be made on each mill each day, the number of samples had to be different because it required the same amount of time to mill seven 2,000-gram samples on the Allis mill as eleven on the Buhler mill. Therefore the comparisons for each day's millings are on the basis of these numbers of samples.

For the benefit of those not familiar with the Buhler mill and because the flow used on the Allis mill may be somewhat different from that used by some workers, the flow sheets of these mills have been shown in Figures 1 and 2.

The Buhler mill, because of its finer adjustments and continuous flow, does not have the errors due to handling of stock that are found in using the Allis mill. The Allis mill, however, has the advantage of the longer and more flexible flow, thus enabling the operator to correct for mistakes made during the milling process. In other words, the errors with the Buhler mill are errors due to the very short flow, where the breaking must be more severe and the grinding harder, which results in a higher-ash, shorter-extraction flour, while the errors with the Allis mill are caused by the handling of the various stocks, necessitated by the discontinuous flow.

The data were analyzed by Fisher's methods and examined particularly for evidences of differentiations between (1) mills, (2) millers, and (3) days.

### Flour Yields, Ash, Protein, and Diastatic Activity

For convenience, the analytical results and the yields of the flours have been presented together in Table I.

The flour yield by the Buhler mill was consistently slightly lower than by the Allis mill. While the difference was significant on only two of the four days, there is little doubt that the Buhler mill because of its short system naturally tends to produce somewhat lower yields of flour than the longer-flow Allis mill. It is interesting to note that despite the lower yield, the ash of the flour is significantly higher.

The protein of the flour was higher in the case of the Buhler in all instances, but the differences were found to be significant only for the first two days' millings. The diastatic activity of the Buhler flours was significantly higher in all instances than that of flours milled on the Allis mill.

In this comparison the variability was greater for the Buhler mill in nearly all instances. From this investigation it may be concluded therefore that experienced millers can replicate milling better on the Allis than on the Buhler mill. The data show no differentiation between the millers, indicating either that they both possess the same skill or that neither mill is likely to differ much in variability of



TABLE I

MEANS AND STANDARD ERRORS OF YIELDS, ASH, PROTEIN AND DIASTATIC ACTIVITY OF ALLIS-CHALMERS AND BUHLER FLOURS

	Day milled	Allis-Chalmers		Buhler	
		Mean	Standard error	Mean	Standard error
Flour Yield Percentage (15% moisture basis)	I	72.9	0.87	72.8	1.45
	II	73.8	1.06 <sup>1</sup>	71.0	1.45
	III	73.5	0.81	72.2	1.76
	IV	73.8	0.56 <sup>1</sup>	72.1	0.76
	Average	73.5	0.82	72.0	1.36
Ash in Flour Percentage (15% moisture basis)	I	0.398	0.014 <sup>1</sup>	0.455	0.016
	II	0.406	0.008 <sup>1</sup>	0.476	0.036
	III	0.399	0.014 <sup>1</sup>	0.435	0.017
	IV	0.396	0.010 <sup>1</sup>	0.453	0.017
	Average	0.400	0.012	0.455	0.022
Protein in Flour Percentage (15% moisture basis)	I	9.81	0.060 <sup>1</sup>	10.05	0.081
	II	9.91	0.052 <sup>1</sup>	10.13	0.098
	III	9.78	0.268	9.83	0.174
	IV	9.61	0.104	9.70	0.168
	Average	9.78	0.121	9.93	0.130
Diastatic Activity (Blish and Sandstedt method)	I	130.0	6.02 <sup>1</sup>	165.0	4.56
	II	127.0	2.27 <sup>1</sup>	171.0	12.25
	III	132.0	10.00 <sup>1</sup>	166.0	8.52
	IV	123.0	5.49 <sup>1</sup>	165.0	6.07
	Average	128.0	5.94	167.0	7.85

<sup>1</sup> The difference between the mills is significant.

performance when handled by trained operators. One would expect that the individuality of the millers would show up more with the less rigidly fixed flow of the Allis mill than with the inflexible flow of the Buhler.

### Discussion

Assuming that the standard errors given in Table I are reasonably good estimates of the replication variability of the two mills, one may compute the magnitude of differences that would be necessary to

TABLE II

DIFFERENCES BETWEEN ANY TWO SINGLE DETERMINATIONS REQUIRED FOR SIGNIFICANCE

	Allis-Chalmers	Buhler
Flour yield, %	2.32	3.85
Ash, %	0.034	0.062
Protein, %	0.34	0.37
Diastatic activity	16.80	22.2

distinguish, definitely, two samples of wheat, or in other words, the significant difference. As it is not customary to replicate experimental millings, the significant differences have been calculated on the basis of single determinations, using the formula: significant difference between single determinations equals  $S.E. \times 2\sqrt{2}$ .

These values are large for both mills, particularly with respect to flour yield and diastatic activity. They indicate that the data on flours produced by single millings ought to be used with a great deal of circumspection.

It should be emphasized that the conditions under which this experiment was conducted were quite variable, especially in respect to temperature and relative humidity of the mill room. However, they were not unlike those occurring in many laboratories and the data herein presented should therefore be useful to the majority of those cereal chemists who have to do experimental milling. Shollenberger (1921), Geddes and West (1930), and others have shown that both temperature and relative humidity need to be accurately controlled if the replication error of the milling procedure is to be reduced to low values.

The estimates of error given in this paper probably represent maximum values to be anticipated with experienced millers working in rooms without control of temperature or humidity. Under the conditions indicated, the Buhler mill certainly exhibits a greater variability than the Allis. Its performance under accurately fixed conditions will be discussed in connection with the second experiment.

Offsetting the disadvantage of poorer replicability is the fact that the Buhler makes an acceptable experimental flour of good extraction, reasonably low ash, and higher diastatic activity than that from the Allis and, above all these considerations, is very much faster in operation.

### Summary of the First Experiment

Seventy-two 2,000-gram samples were milled from one uniform lot of wheat, in a comparison of the replicability of the Allis-Chalmers and the Buhler experimental mills, by two operators, each having had wide experience with each mill.

The variability of the Buhler was higher in practically all cases than that of the Allis. There was very little evidence of differences in variability between millers. The Buhler mill is easier and faster to operate than the Allis.

### Second Experiment, with Controlled Temperature

Shortly after the foregoing experiment was completed, air-conditioning equipment was installed in the mill room, and it was thought

advisable to conduct another test of the two mills under controlled temperature conditions. The experiment as performed differed somewhat from the plan of the former one. Two varieties of wheat were used, and three millers participated. One miller was highly experienced, one had had a small amount of experience with each mill, while the third had had no routine experience whatsoever.<sup>5</sup> Each operator alternated on the mills. As in the former experiment, different numbers of samples were milled on each mill because the Buhler mill is more rapid in operation than the Allis mill; ten samples per day were milled on the former and six on the latter. In each day's milling half the samples were of each of the two varieties. The general plan of the experiment can be seen from Table III in which is presented the average flour extraction, protein, and ash contents.

TABLE III  
MEANS OF FLOUR EXTRACTION, PROTEIN, AND ASH CONTENT

Variety	Buhler mill—Miller				Allis mill—Miller			
	X	Y	Z	Av.	X	Y	Z	Av.
	Flour Extraction <sup>1</sup> %							
B	72.3	73.4	70.1	71.9	70.4	69.5	68.8	69.6
C	75.8	77.0	75.1	76.0	69.8	71.3	71.0	70.7
	Protein Content <sup>1</sup> %							
B	13.4	13.0	12.7	13.0	13.2	12.9	12.3	12.8
C	12.5	12.2	12.1	12.3	12.1	12.2	11.9	12.1
	Ash Content <sup>1</sup> %							
B	0.47	0.45	0.40	0.44	0.39	0.39	0.37	0.38
C	0.49	0.45	0.41	0.45	0.38	0.39	0.38	0.38

<sup>1</sup> Moisture basis 13½%.

Considering the averages given in Table III the most striking thing is the greater differentiation obtained between varieties B and C with the Buhler mill than with the Allis mill. With the Buhler, all three operators obtained significantly higher flour yields with variety C than with B; on the other hand there was no appreciable difference between these two wheats when milled on the Allis mill.

Table IV gives the standard errors of replication for the second experiment. As might be expected some of the errors are larger than in the previous experiment. On the other hand, it is noticeable that there is very little differentiation between the two mills, with both experienced and inexperienced operators. The only instance of significance was in the case of the flour protein.

As a final check on the results of the second experiment bakings were made of each variety milled by each miller on each mill the

<sup>5</sup> Each miller had a sound fundamental knowledge of the principles involved but his experience in using the mills with different wheat varieties was as stated above.

TABLE IV

STANDARD ERRORS OF REPLICATION OF FLOUR EXTRACTION, FLOUR PROTEIN, AND FLOUR ASH, WITH BOTH EXPERIENCED AND INEXPERIENCED MILLERS

	Buhler mill—Miller				Allis mill—Miller			
	X	Y	Z	Av.	X	Y	Z	Av.
Flour extraction, <sup>1</sup> %	1.5	0.8	0.8	1.0	1.3	1.6	0.7	1.2
Flour protein, <sup>1</sup> %	0.25	0.21	0.38	0.28	0.21	0.18	0.10 <sup>2</sup>	0.15
Flour ash, <sup>1</sup> %	0.019	0.008	0.014	0.013	0.013	0.006	0.013	0.011

<sup>1</sup> Moisture basis 13½%.<sup>2</sup> The difference between mills is significant.

second day. Only the samples that represented the high and low extractions for the day were baked. The flours were baked unbleached but had been matured by storage at room temperature.

The following commercial-type straight-dough formula was used:

	Grams	Percentage based on flour
Flour	200 (15% m.b.)	100
Water	As required	...
Sugar	10	5.0
Salt	3	1.5
Yeast	4	2.0
Shortening	6	3.0
Dry skim milk	8	4.0
Potassium bromate	0.002	0.001

The doughs were mixed to optimum development with the Swanson mixer, divided into duplicate loaves, fermented at 30° C. for 3 hours (105 minutes to the first punch, 50 minutes to the second punch and 25 minutes to the pan). They were proofed at 30° C. for 55 minutes and baked for 25 minutes at 420° F.

The baking data obtained are presented in Table V. There was no appreciable difference in baking quality of the flour from either mill or from any of the millers on any one mill, which may be due in part to the fact that both varieties had very poor baking qualities.

### Summary of Second Experiment

Ten samples of each variety were milled by each of three millers (one experienced, one slightly experienced, and one inexperienced) on the Buhler mill and six samples of each variety on the Allis mill.

The flours were examined with respect to the percentage of flour extraction ascribable to millers and mills. On the Allis mill the experimental error more than covered differences of extractions between varieties. There was no significant difference due to either miller or mill as far as baking quality was concerned, which may be due in part to the extremely poor baking quality of both varieties.

TABLE V  
BAKING DATA ON FLOURS FROM BUHLER AND ALLIS-CHALMERS MILLS<sup>1</sup>—  
2000-GRAM SAMPLES

Miller	Sample B				Sample C			
	Flour extrac- tion	Water absorp- tion	Grain and texture score	Loaf volume	Flour extrac- tion	Water absorp- tion	Grain and texture score	Loaf volume
	%	%		cc.	%	%		cc.
Buhler Mill								
X	72.1	62	75F+	700	77.5	59	55VP	570
X	70.3	62	75F+	690	75.6	59	55VP	555
Y	73.2	63	70F+	675	76.8	58	55VP	535
Y	71.5	63	75F+	685	76.3	60	55VP	555
Z	70.2	62	75F+	690	73.4	57	55VP	560
Z	70.0	61	80F+	670	73.5	57	55VP	555
Allis-Chalmers Mill								
X	72.6	62	75F+	700	68.4	57	55VP	545
X	68.8	63	80F+	695	70.1	58	55VP	540
Y	72.5	61	75F+	715	73.1	58	55VP	555
Y	70.8	60	80F+	710	71.6	60	55VP	565
Z	68.0	60	80F+	670	71.4	56	55VP	535
Z	69.0	61	80F+	680	70.8	57	55VP	550

<sup>1</sup> These data were secured from K. F. Finney, Baking Technologist, Hard Winter Wheat Quality Laboratory.

### General Summary and Conclusions

The following general conclusions seem to be justified:

The variabilities of yield, ash, and diastatic activity are greater for the Buhler mill than for the Allis mill when the millers are skilled operators and the atmospheric conditions of the mill room are not controlled. These differences although not great are significant.

With the milling room air conditioned, and with both experienced and inexperienced millers, there were no significant differences between mills or millers, except with respect to flour protein, where one miller had better results on the Allis mill.

On the other hand, differences between wheats of different milling quality show up clearly with the Buhler mill when they may not be detectable by means of the Allis mill. Whatever the differences between the flours produced on the two mills, they were not great enough to be detected by the baking test.

The greater speed and ease of operation of the Buhler mill, together with its very compact construction, commend it to cereal technologists, especially where the volume of routine work is large.

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## A COMPARISON BETWEEN THE ALLIS-CHALMERS AND MICRO-MILLING TECHNIQUES ON NORTH DAKOTA HARD RED SPRING WHEATS

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A description of a small or micro experimental flour mill suitable for the production of satisfactory flour from relatively small samples of wheat was reported by Geddes and Frisell (1935). This mill was designed to fulfill the need for a method of producing flour comparable in character to that yielded by the regular experimental mill but which would require a relatively small sample of wheat. By the use of this technique, in conjunction with a baking procedure which would yield miniature loaves and require a small quantity of flour, tests of milling and baking quality could be made available much earlier in the wheat-breeding program than had been possible with the older, standard experimental methods.

A study of the chemical attributes and baking quality of flours milled by the two procedures from the same series of 28 wheats of widely different protein content and baking strength was made by Geddes and Aitken (1935). The smaller mill was found to give a lower yield of flour and lower recovery of total products, but the flours were equal to those produced by the Allis mill in protein content. The micro-mill flours were higher in ash and carotene content, and slightly higher in diastatic activity. The milling losses were 3.4% and 1.0% respectively. Correlation coefficients for flour yield, protein,

ash, carotene, diastatic activity, and loaf volume for the flours produced by the two milling methods were very high and showed marked significant relationships for these factors in the two sets of flour. Difficulty was experienced, however, in scoring internal factors with the miniature loaves.

Harris and Sanderson (1938) investigated the merits of the miniature 25-gram baking procedure as compared with the 100-gram method. A series of 76 flours milled on the Allis-Chalmers experimental mill was employed as experimental material. Significant positive correlations were found between wheat protein and loaf volume for the two baking methods, and a relatively high positive correlation of  $+ .8068$  between the loaf volumes produced by the two methods. The authors concluded that this relationship would be useful in differentiating between strong and weak wheat varieties, but postulated that the surface of the cut loaf was too small to score satisfactorily for crumb color and texture.

VanScoyk (1937, 1939) found that the micro-baking technique gave as informative data as the larger-loaf procedure. Mechanical molding equipment was employed and no difficulty was experienced in obtaining satisfactory differentiation of both external and internal characteristics.

A special grant by the North Dakota Legislature in 1937 for the purchase of milling equipment made possible the purchase of a micro mill of similar construction to the one used by Geddes, Frisell, and Aitken. It was deemed advisable to investigate a series of flours milled from hard red spring wheats, grown in North Dakota, by the older Allis-Chalmers experimental mill, and to compare their chemical and baking characteristics with a similar series milled from the same wheats by the micro technique. In this way, knowledge regarding the relative performance of the two mills on North Dakota wheat would be gained, thereby indicating the suitability of the new micro technique in evaluating new wheats for the plant breeders.

### Experimental Material and Methods

A group of 26 samples, each one of which contained a sufficient quantity of hard red spring wheat for millings on both the Allis-Chalmers and the micro mill, was selected for the purposes of this investigation. These wheats were free from damage of any kind, and graded relatively high, only one sample grading lower than No. 1 dark northern spring. The samples were cleaned, scoured, and tempered to a moisture content of 15% previous to milling. The milling techniques employed were very similar for both mills, with the exception that smaller quantities of wheat were milled in the instance of the

micro mill, and that only one corrugated roll was available, in this instance, as compared with the two sets of corrugated rolls in the Allis set-up.

The resultant flours from both mills were analyzed for moisture, protein, and ash, and for diastatic activity. The flours were baked by the micro procedure with the malt-phosphate-bromate formula, as this method has been shown to give maximum differentiation between samples. The moisture determinations were made in a Freas electric oven at  $130^{\circ} \pm 3^{\circ}\text{C.}$  for one hour. The ash was run by the magnesium acetate method in an electric muffle at  $750^{\circ}\text{C.}$  for one hour. The Blish-Sandstedt ferricyanide method as adapted by Sandstedt (1937) was used to determine the diastatic activity.

### Discussion

The grades, test weight per bushel, wheat protein, flour yields, and total recovery of products are shown in Table I. The test weight of

TABLE I  
GRADE, TEST WEIGHT, WHEAT PROTEIN AND MILLING DATA  
Arranged in Order of Increasing Wheat Protein

Lab. No.	Grade <sup>1</sup>	Test weight	Wheat protein <sup>2</sup> (N $\times$ 5.7)	Flour yield		Total recovery of products <sup>2</sup>	
				Allis	Micro	Allis	Micro
		<i>Lbs. per bu.</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>
11	2 DNS	57.4	13.3	72.1	73.0	99.0	99.3
14	1 HDNS	61.0	13.4	75.3	77.0	98.6	98.4
13	1 HDNS	60.6	13.5	75.8	77.8	99.0	99.6
9	1 HDNS	62.0	13.6	78.4	77.5	99.0	98.3
20	1 DNS	59.5	13.7	74.3	72.5	98.8	98.3
12	1 HDNS	60.7	13.8	75.4	76.9	99.1	99.5
22	1 HDNS	61.3	13.8	76.3	75.4	99.3	100.2
1	1 DNS	58.6	13.9	75.0	70.4	98.6	97.0
15	1 DNS	59.5	13.9	77.1	74.7	99.1	97.6
18	1 HDNS	60.8	14.1	77.2	77.8	99.1	98.3
4	1 DNS	59.6	14.2	75.7	74.1	99.1	97.4
5	1 HDNS	60.4	14.2	75.0	72.0	99.1	98.4
19	1 HDNS	60.3	14.2	75.6	75.1	99.6	97.8
16	1 DNS	59.3	14.3	76.7	77.2	99.7	99.7
21	1 HDNS	62.1	14.3	76.4	74.3	99.2	98.0
3	1 HDNS	60.6	14.4	76.1	74.0	98.6	100.2
7	1 HDNS	60.8	14.4	76.2	77.0	100.2	98.8
23	1 HDNS	61.5	14.4	76.0	75.8	99.8	97.4
10	1 HDNS	62.0	14.5	78.0	78.1	99.3	96.1
17	1 HDNS	61.2	14.5	77.9	77.4	99.8	99.1
25	1 HDNS	62.5	14.5	77.3	74.3	99.6	97.3
2	1 HDNS	62.0	14.6	75.9	72.5	99.1	97.5
8	1 HDNS	60.6	14.8	75.9	72.5	98.9	95.1
24	1 HDNS	62.0	14.8	76.1	76.3	98.8	98.9
6	1 HDNS	60.4	15.4	73.1	71.7	97.8	97.6
26	1 HDNS	60.0	17.0	74.6	75.0	99.1	98.2

<sup>1</sup> Unofficial.

<sup>2</sup> Moisture basis 13.5%.



these wheats ranged from 57.4 to 62.5, and the wheat protein from 13.3% to 17.0%. There was thus a small variation in test weight, but a more substantial difference in wheat protein. The flour yield varied from 72.1% to 78.4%, a range of 6.3% for the Allis-Chalmers experimental mill, and from 70.4% to 78.1%, a range of 7.7% in the case of the micro mill. In percentage of total recovery of products the values varied from 97.8% to 100.2% for the Allis, and from 95.1% to 100.2% for the micro mill. The corresponding ranges are 2.4% and 5.1% respectively. There accordingly appears to be greater variability in the yields obtained on the smaller unit, a conclusion borne out by the flour yield values, and the work of Geddes and Aitken (1935).

In Table II are shown the comparative data obtained from an analysis of the two series of flours. It is probable that the moisture percentages were not very pertinent, as the wheats were tempered in different lots for each mill, and accordingly these values are not given. The flour protein percentages appear quite similar, but differences are

TABLE II  
COMPARATIVE DATA OBTAINED FROM THE TWO SERIES OF FLOURS  
Arranged in Order of Increasing Wheat Protein

Lab. No.	Ab-sorp-tion	Loaf volume		Protein (N×5.7) <sup>1</sup>		Ash <sup>1</sup>		Diastatic activity <sup>1</sup>	
		Allis	Micro	Allis	Micro	Allis	Micro	Allis	Micro
	%	cc.	cc.	%	%	%	%	%	%
11	60	154	142	12.5	12.7	0.51	0.72	117.9	160.0
14	60	133	145	12.5	13.2	.50	.67	122.1	151.5
13	62	136	150	12.6	12.8	.54	.76	139.8	167.8
9	60	137	148	13.1	12.9	.54	.71	111.5	124.8
20	60	162	162	12.6	13.3	.45	.67	97.2	115.4
12	60	148	146	12.7	13.3	.54	.74	90.7	111.2
22	61	142	136	13.0	13.6	.65	.63	143.3	185.6
1	62	136	151	13.4	13.2	.47	.65	103.9	106.6
15	57	128	133	12.5	13.2	.50	.67	91.2	124.5
18	62	132	140	13.1	13.3	.54	.72	134.0	179.6
4	65	132	139	13.4	13.4	.53	.81	128.1	161.4
5	64	122	139	13.5	13.7	.61	.80	145.0	172.8
19	60	117	125	13.2	13.4	.52	.72	146.7	177.2
16	60	145	138	13.0	13.2	.53	.65	88.6	114.3
21	60	162	145	13.1	13.4	.50	.73	97.0	125.6
3	61	127	142	13.6	13.3	.52	.66	100.3	117.4
7	60	144	156	13.5	13.9	.39	.72	87.5	110.2
23	60	148	130	13.3	13.9	.51	.83	119.7	167.7
10	62	137	137	12.6	14.0	.58	.84	136.2	194.3
17	60	148	159	14.0	13.8	.52	.75	115.1	153.4
25	60	148	148	13.5	13.6	.68	.68	102.3	132.6
2	61	127	172	13.4	13.8	.54	.73	94.0	123.2
8	60	145	145	13.5	13.7	.37	.68	88.4	120.2
24	60	165	138	13.5	14.0	.41	.65	107.4	133.4
6	59	111	118	14.5	14.6	.41	.69	79.0	99.4
26	60	170	154	16.2	16.2	.42	.84	99.5	140.3

<sup>1</sup> Moisture basis 13.5%.

apparent in ash and diastatic activity between the flours produced by the two milling procedures.

Table III shows the means, differences between means, standard deviations, and coefficients of variability computed from the data. The same absorption was shown for each pair of flours regardless of the milling method. With the exception of protein and loaf volume, the micro-mill results appear to be more variable than the Allis values. The differences between mean flour protein, flour yield, and loaf volume are clearly not significant. Flour ash and diastatic activity are

TABLE III  
TABLE OF STATISTICAL CONSTANTS  
Means, Standard Deviations and Coefficients of Variability

	Means			Standard deviations		Coefficients of variability	
	Allis	Micro	Difference <sup>1</sup>	Allis	Micro	Allis	Micro
Flour protein %	13.30	13.58	0.28	0.752	0.676	5.65	4.98
Flour yield %	75.90	75.01	0.89	1.385	2.174	1.82	2.90
Total recovery %	99.13	98.23	0.90	0.473	1.174	4.77	11.95
Flour ash %	0.51	0.72	0.21	0.073	0.195	14.31	27.08
Diastatic activity	111.02	141.17	30.15	20.07	27.343	18.08	19.37
Loaf volume cc.	140.62	143.77	3.15	14.422	11.288	10.26	7.85
Moisture %	14.35	13.96	0.38	0.282	0.299	1.96	2.14

<sup>1</sup> Significant differences are shown in heavier type.

significantly higher for the micro flours. The same tendency was noted by Geddes and Aitken (1935), although their mean differences were not found to be significant in the case of diastatic activity. In the present investigation, it is extremely probable that sharper rolls on the new micro mill as compared with the duller rolls on the Allis, which had been in use over a comparatively long period, materially increased the spread between these means. Moisture content of flour and total recovery of products were higher for the Allis procedure. Geddes and Aitken found that the micro method had a higher loss, and therefore lower total recovery, than the Allis procedure.

The correlation coefficients calculated from the data are shown in Table IV. Significant positive correlations between flour protein, flour yield, diastatic activity, and loaf volume are demonstrated, although the relationship between the two series of loaf volumes is lower than one would expect from a knowledge of the results obtained in a similar investigation conducted by Geddes and Aitken (1935), and of the conclusions reached by Harris and Sanderson (1938). It must be borne in mind, however, that Geddes and Aitken worked with wheats of widely different protein content and baking strengths, whereas the

TABLE IV  
CORRELATION COEFFICIENTS COMPUTED FROM THE COMPARATIVE DATA

Variables correlated		Correlation coefficient	$P^1$
$X$	$Y$	$r_{xy}$	
Flour protein %	Flour protein %	<b>+ .8815</b> <sup>2</sup>	<.0001
Flour yield %	Flour yield %	<b>+ .6075</b>	.0010
Total recovery %	Total recovery %	<b>+ .0914</b>	>.5542
Flour ash %	Flour ash %	<b>+ .0325</b>	>.5542
Diastatic activity	Diastatic activity	<b>+ .9263</b>	<.0001
Loaf volume (Allis) cc.	Loaf volume (micro) cc.	<b>+ .4025</b>	.0420
Test weight	Flour yield (Allis-Chalmers) %	<b>+ .6811</b>	.0002
Test weight	Flour yield (micro) %	<b>+ .4221</b>	.0318

<sup>1</sup> Probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.

<sup>2</sup> Significant correlation coefficients are shown in heavier type.

wheats employed in the present investigation all belonged to the hard red spring class, and did not vary widely in strength. Harris and Sanderson used only a series of flours milled on the Allis-Chalmers, so the results reported by these two pairs of independent investigators are not strictly comparable to the data contained in this study. A summary of the color and texture scores revealed little difference between the two methods in respect to these characteristics.

### Summary and Conclusions

A series of 26 samples of hard red spring wheat were milled into straight flour by two experimental procedures. One was the usual method, using 2,000 grams of wheat which was milled in an Allis-Chalmers mill consisting of two corrugated rolls and one smooth roll, while the other was the micro-milling procedure described by Geddes and Frisell (1935). The resultant flours were analyzed for protein, ash, moisture, and diastatic activity, and were baked into miniature loaves employing doughs made from 25 grams of flour.

An analysis of the data showed no significant differences between the mean values of the two series of flours in respect to protein content, flour yield, and loaf volume. Significant differences in means, however, were shown for flour ash and diastatic activity, the micro-milled flour being highest in each instance. It is extremely probable that the sharper rolls on the micro mill, as compared with the Allis, affected these flour characteristics.

Significant positive correlation between milling methods was demonstrated for flour protein, flour yield, diastatic activity, and loaf volume, although the majority of the coefficients were not sufficiently high to justify predicting one variable from a knowledge of the other. It is probable that a wider range in flour strength would increase the correlation between loaf volumes.

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## VARIATIONS IN DOUGH-DEVELOPMENT CURVES

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The use of physical methods, particularly recording dough mixers, in the testing of wheat and flour is restricted in comparison with the use of chemical determinations and the baking tests. While the users of chemical and baking tests are numbered in the hundreds, the number of users of recording dough mixers is limited, at least in North America, to a few dozen. Greater progress has been made in perfecting the mechanical devices for making these physical tests than in the interpretation of their results in terms of baking value. One of the reasons for the slowness in learning the interpretation of these dough-development curves is the comparatively small number who use the curves and the consequent limited exchange of ideas concerning their meaning in practical use. Another difficulty is the lack of an adequate standard for interpreting these curves.

### Studies in Dough Development

The watt meter was used by Swanson and Working (1933) in recording the behavior of dough during development. St. John and Bailey (1929) attached a watt meter to a dough-mixing machine and by this means measured the power requirements for mixing. These authors used Bingham and Murray's (1923) method in measuring the

<sup>1</sup> Contribution No. 59, Department of Milling Industry.

plasticity. The relationship of mixing speed to dough development was studied by Stamberg and Bailey (1938), who state that a medium to moderately high speed resulted in superior bread. Halton and Scott Blair (1937) studied physical properties of flour in relation to their bread-making qualities and they state that the two properties which are of chief importance are viscosity and elasticity modulus. Malloch (1938) devised a recording mixer which operates on flour samples containing only 7 grams of dry matter. The curves obtained showed a break in curvature which varied in position and sharpness for the different flours. There were indications that the time of the break is related to gluten quality. Bohn and Bailey (1936) used a "stress meter" to study the stress-strain relation of flour dough, "including a study of plastic flow by measuring the dying out of stress with time of stretching the dough." It was found that strong flours gave higher stress readings than weak flours and that high stress-meter readings are a good indication of the ability of dough to withstand prolonged mixing. Schofield and Scott Blair (1932, 1933, and 1933) studied the relationship between viscosity, elasticity, and plastic strength of dough, using an apparatus by which a piece of dough could be stretched for varying lengths of time, and also noted the dying-out stress.

### Hogarth's Device

The knowledge of a mechanism for testing and recording the physical properties of flour existed about the time cereal laboratories were first started in connection with flour mills in the United States. An application for a patent on such a device was filed by J. Hogarth in the United States patent office in 1890 and the patent was granted May 10, 1892, letters patent No. 474,636. It is stated that this invention has appliances for mechanically testing and sampling different qualities of flour and graphically indicating and recording the various characteristics or properties of the different flours tested. The dough was mixed in a kneader of the Werner-Pfleiderer type and the resistance of the dough was recorded on cross-section paper using the dynamometer principle. There was also provision for determining the amount of water required to produce a dough of desired consistency. Cereal chemists are still looking for a satisfactory method of measuring water absorption.

### Extensimeters

Hogarth's device apparently did not become well known since it does not seem to be mentioned in the literature of the cereal chemists.<sup>2</sup> The extensimeter designed by Chopin (Bailey and Le Vesconte, 1924;

<sup>2</sup>The author is indebted to Dr. C. H. Bailey for calling attention to Hogarth's invention

Chopin, 1927) measures the tensile strength of a sheet of dough when blown into a bubble by means of expanding air under accurately controlled pressure. The Comparator, made by Buhler Brothers, Switzerland, is also a device to measure the tensile strength of a sheet of dough when subjected to the pressure exerted by a rounded, truncated cone. Kress (1924) reports a device by James to record in a curve the resistance of wet gluten to stretching, the distance it can be stretched before breaking, and the character of the break whether gradual or sharp. Hankóczy (1920) used this principle on the wet crude gluten. The ideas underlying these devices were that the volume and behavior of the bubble were related to the baking strength of the flour. Other references to physical methods are given by Swanson (1938).

### Recording Dough Mixer

The farinograph (Brabender 1932, 1934) embodies several ideas found in Hogarth's device. Hankóczy developed a machine for determining the physical properties of dough in order to have a laboratory method which would give information on the qualities of the Hungarian wheat crop. He needed especially a device to determine absorption.<sup>3</sup> The Swanson-Working dough mixer (Swanson and Working, 1933) was designed to measure and record automatically the rate of dough development, the duration of resistance against mechanical action, and the rate and extent of increase in mobility of dough as a result of mechanical action. That these characteristics are related to inheritance was shown by Swanson (1936) and in so far as this is true, the dough-mixer curves serve one of the primary needs of wheat improvement work, namely, to differentiate characteristics due to inheritance. The distinctive and, as far as known, original feature of the recording dough mixer is the principle of mixing. As soon as the water films on the flour particles have become uniformly distributed in the dough so as to develop the elastic, plastic, and viscous properties, the dough is subjected to a continuous stretching, folding, and restretching action. This subjects a small body of dough to a treatment which is similar to that given in large mixers where the force of gravity serves the same function as the fixed pins in the bowl of the recording mixer, namely, to hold the dough back while being stretched and folded by the upper pins which have a planetary motion.

### Mobility in Dough Structure

The characteristics of the curves depend on the condition of the mobility of dough constituents in relation to each other and the

<sup>3</sup> Private communication.

changes in this mobility during the progress of mixing. This mobility is influenced by the rate of wetting or hydration of the flour particles, the development and orientation of the gluten strands, and the conditions which influence plasticity or the movement or sliding of particles relative to each other. There is usually at first a gradually increasing resistance to the movement of the revolving pins and the amount of this resistance is related to the height of the curve. Continued mixing beyond the point of maximum resistance generally causes an increase in the mobility. This may be due either to the breaking of the gluten strands or to the disintegration of the colloidal complex. Markley and Bailey (1938) conclude that "the use of this rate of increase in mobility upon prolonged mixing is impractical as a simple and direct measure of flour strength, since it is the resultant of so many variable factors."

### Curve Characteristics

Experience with pure varieties of different types has shown that the curve characteristics most closely related to baking phenomena are steepness of the ascending slope, the time required to reach the peak, the character of this peak whether sharp or rounded, and the general width and height of the curve. The steepness of the descending slope and the narrowing of the curve due to increased mobility, which to a certain extent indicates the rate and extent of the breakdown of the dough structure, have also a value. Since mixing for baking usually is not carried to the point of increased mobility in dough, this can have only a theoretical value as an indication of inherent dough structure. The time between any important points in the curves presented in the accompanying illustrations can be estimated. The distance between the curved lines of the graphs represents three minutes of elapsed time.

It should be clearly understood that curve characteristics from different recording mixers will not be similar unless the adjustments and conditions of operation are the same. How much curve characteristics may differ simply as a result of adjustments can be seen by comparing the curves shown by Swanson (1936) with those shown by Swanson and Clark (1936) as well as by Swanson (1938). Absorption or the amount of water used in mixing the dough has also important effects. Thus curves made in different laboratories should not be compared at least in minor details unless it is known that the adjustments and conditions of operation are also comparable. This situation adds another problem in cooperative work with dough mixer curves.

### Variations in Curves

In the previous papers (Swanson and Working, 1933; Swanson, 1936) the possibilities of using the curves as indicating characteristics due to heredity were stressed. In the continued use of the curves in connection with wheat-variety studies it has become evident that while the curves of various varieties follow a certain pattern, there may be very wide variations which must be recognized. Thus it has been found that different varieties may produce curves which are as much alike as if they were from the same variety, and curves from the same variety may vary as much as curves from different varieties. This is shown in the figures presented.

### Causes of the Variation

The variations from a general type or pattern are due to weather conditions, particularly during heading and ripening, and the protein content. The protein content has a marked influence on the height and general shape of the curve. The main point in this paper is to show how much and in what respects dough curves may be influenced by environmental factors when wheats are grown in various places and in different years. The curves selected for this study were from three crop years, 1935, 1936, and 1937. These years represented wide variations in weather conditions, both annually and locally. The year 1936 was extremely hot and dry in Kansas. The other two years were more nearly normal.

It should be clearly understood that these curves were not made as part of a planned experiment but were selected from tests made on varieties grown in the three years. All of the curves selected for illustration and study were from wheats grown in Kansas except the four spring varieties which were included for comparison.

### General Plan for Presentation of Curves

The general plan followed in selecting the curves presented in this paper was first to find one or more curves from each of the varieties mentioned below which had the general pattern characteristic of most curves obtained on that variety. This pattern is based on the mental picture obtained from the study of a large number of curves from the various varieties, and it means that most of the wheats of average protein content and grown under normal weather conditions will give curves which are similar for a particular variety. Next, curves were selected which showed the greatest deviation from this general pattern. Besides these, curves were chosen to represent various intermediate types. These groups of curves were then mounted and photographed.



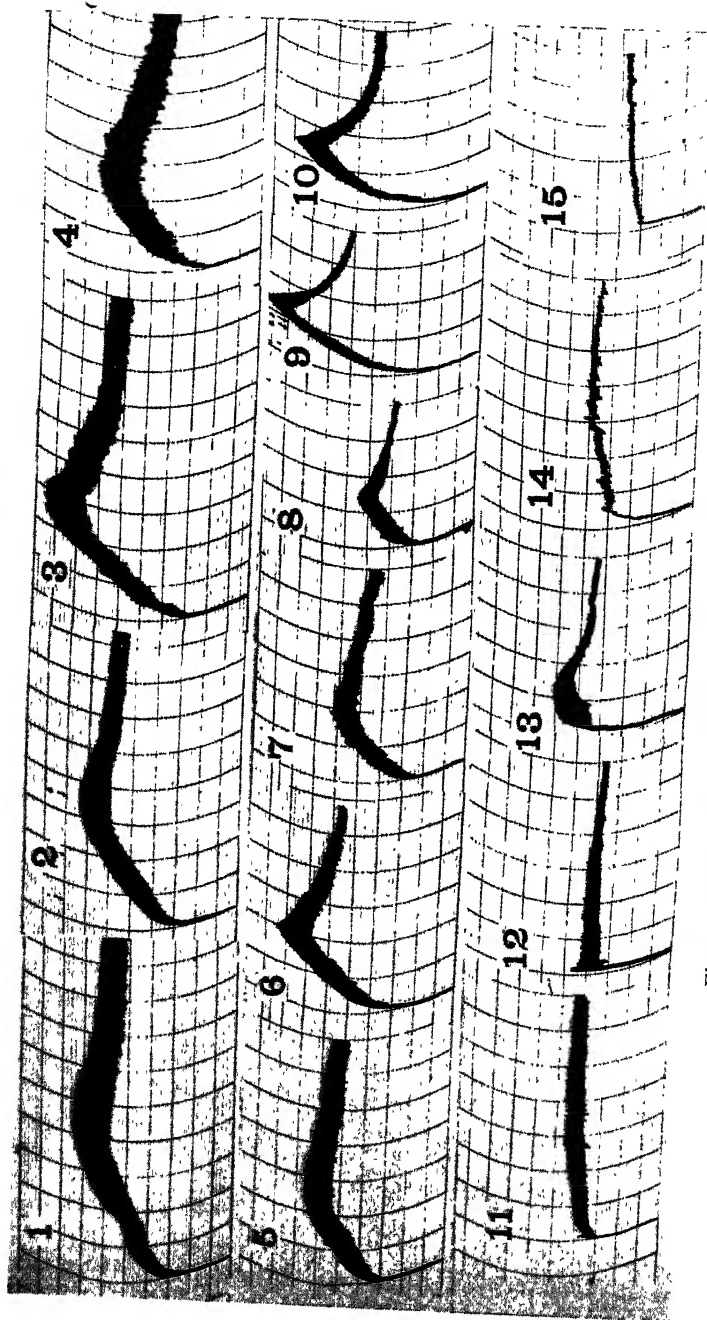


Fig. 1. Group of curves showing a wide range in characteristics.

The curves from the different varieties which showed the widest differences were chosen.

The following varieties are represented in Figures 2 to 9.

*Hard Red Winter Wheats*

Turkey  
Kanred  
Tenmarq  
Cheyenne

Blackhull  
Chiefkan  
Early Blackhull

*Hard Red Spring Wheats*

Marquis  
Ceres  
Reward  
Thatcher

*Soft Red Winter Wheats*

Fulcaster  
Kawvale (semi hard)  
Harvest Queen  
Clarkan

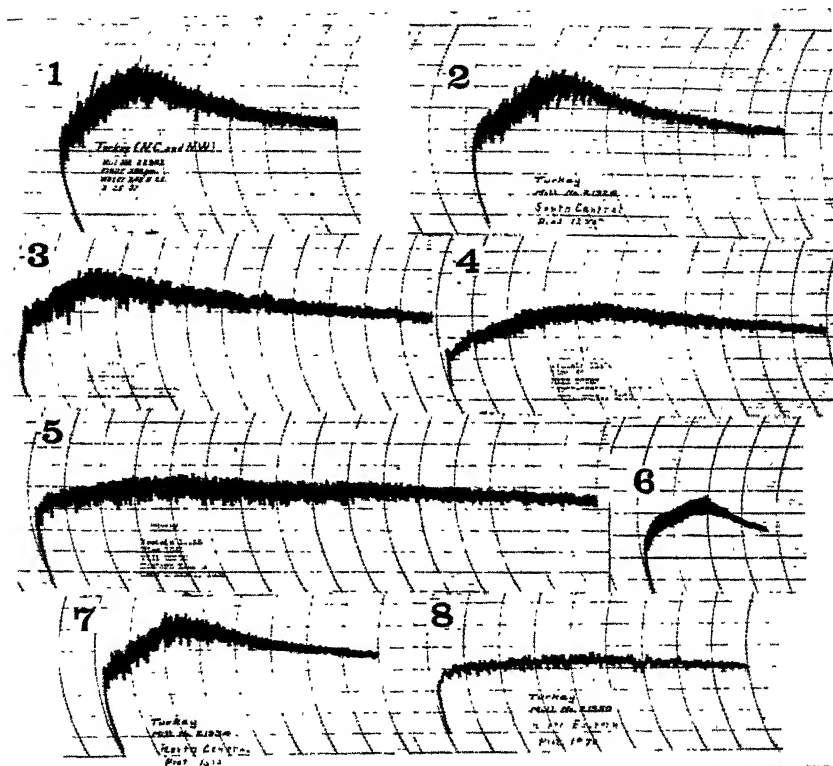


Fig. 2. Turkey flours.

No.	Sample	Place grown in Kansas	Year	Test weight Lbs.
1	22903	N. Central and N.W.	1937	59.2
2	21926	S. Central	1936	58.9
3	20555	S. Central	1935	56.3
4	20563	N. Central	1935	55.9
5	20572	Eastern	1935	56.8
6	22008	Hays Exp. Sta. (N. Central)	1936	59.2
7	21934	N. Central	1936	57.0
8	21950	N. East	1936	59.5

### Main Curve Characteristics as Illustrated in Figure 1

In Figure 1 are presented various types of curves without any reference to variety. These 15 curves show the range of variations which are shown in the other figures presenting the curves of the various varieties. The four main characteristics in the curves are:

1. The steepness and length of the ascending slope, which indicates the rate and manner of dough development.

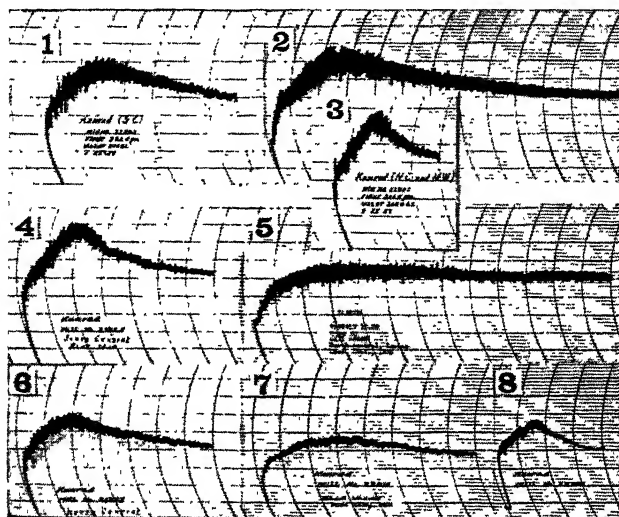


Fig. 3. Kanred flours.

No.	Sample	Place grown in Kansas	Year	Test weight Lbs.
1	22893	S. Central	1937	60.6
2	20554	S. Central	1935	54.6
3	22902	N. Central and N.W.	1937	58.7
4	21925	S. Central	1936	58.3
5	20562	N. Central	1935	54.8
6	21933	N. Central	1936	60.9
7	22010	Northwest	1936	52.5
8	22006	Hays Exp. Sta. (N. Central)	1936	58.6

2. The general shape of the turn at the top, which shows the duration of time during which the dough maintains its resistance against the mechanical force.

3. The general width of the curve, which is an indication of the elastic properties.

4. The steepness of the descending slope and the amount of narrowing of the curve, which indicate the rate and the extent of increase in the mobility.

### Rate of Dough Development

The rate of dough development may be characterized as very slow in curves 1 and 2; slow in curves 3, 4, and 5; medium in curves 6, 7, and 8; rapid in curves 9 and 10; and very rapid in curves 12 and 13. The rate of dough development seems to be associated with the rate of wetting or hydration. Curves like 9 and 10 show a rapidly increasing resistance as the dough develops, while in curves like 11, 12, and 13 there is little or no increase in resistance after the dough begins to form. Doughs like 1 and 2 behave as though the gluten strands were longer and hence become arranged in a parallel pattern more slowly. The increase in resistance apparently takes place as

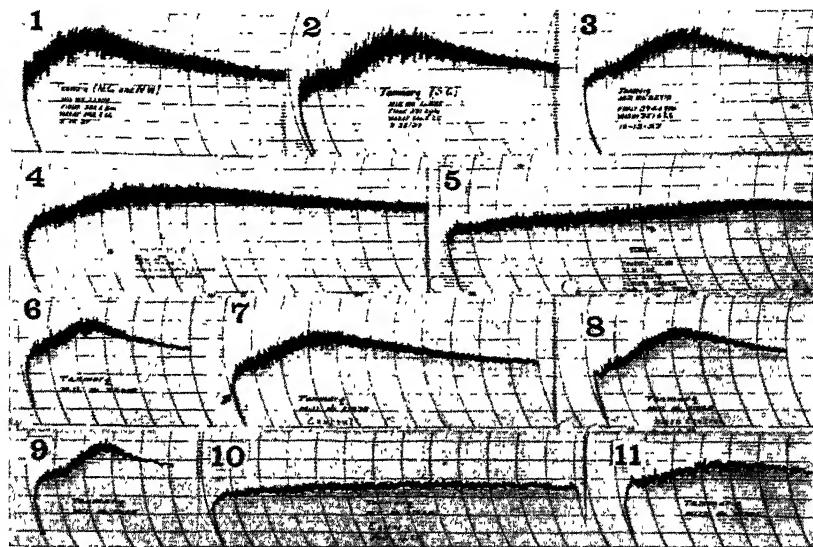


Fig. 4. Tenmarq flours.

No.	Sample	Place grown in Kansas	Year	Test weight Lbs.
1	22904	N. Central and N.W.	1937	58.8
2	22895	S. Central	1937	
3	22910	Central	1937	59.4
4	20564	N. Central	1935	56.9
5	20573	Eastern	1935	54.5
6	22009	Hays Exp. Sta. (N. Central)	1936	58.4
7	21938	Central	1936	57.5
8	21935	N. Central	1936	56.5
9	22005	Hays Exp. Sta. (N. Central)	1936	60.3
10	21944	Eastern	1936	57.9
11	21991	Manhattan (E. Central)	1936	58.8

more and more of these strands are oriented. At the point of maximum orientation in curves like 9 and 10, the dough begins to break rapidly. In curve 12, the wetting is almost immediate and in 11 and 13 very rapid, which means that the elastic properties of the dough are developed almost at once. Low and thin curves like 14 and 15 show a lack of dough development and the behavior is similar to that of soft putty. The plastic properties are so prominent as to submerge the elastic. Curves like 14 and 15 indicate a pronounced abnormal

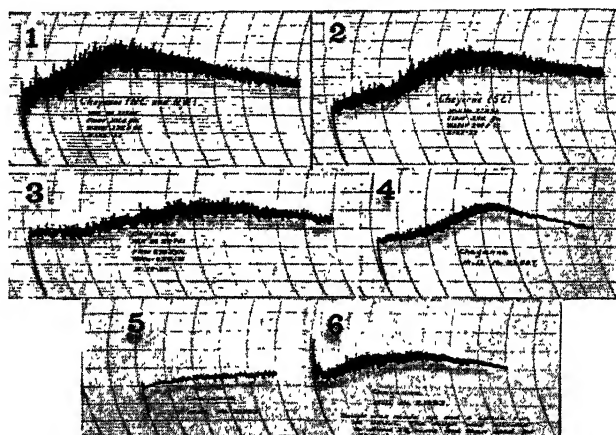


Fig. 5. Cheyenne flours.

No.	Sample	Place grown in Kansas	Year	Test weight Lbs.
1	22905	N. Central and N.W.	1937	59.5
2	22896	S. Central	1937	60.5
3	22741	Manhattan (East Central)	1937	60.8
4	22007	Hays Exp. Sta. (N. Central)	1936	60.4
5	21993	Manhattan (East Central)	1936	59.6
6	21993	(Made after 4 hours in dough)	1936	59.6

condition, while curves like 11, 12, and 13 may be obtained from low-protein flours, 11 is from a low-protein hard wheat, and 12 and 13 are from soft wheats.

### Top Characteristics

A very rounded top is represented by curves 1, 2, and 5, rounded by 3, 4, and 7, while 9 and 10 have a sharp top. A sharp top is generally accompanied by a rapid dough development showing that the stiffness increases very rapidly, but the duration of maximum

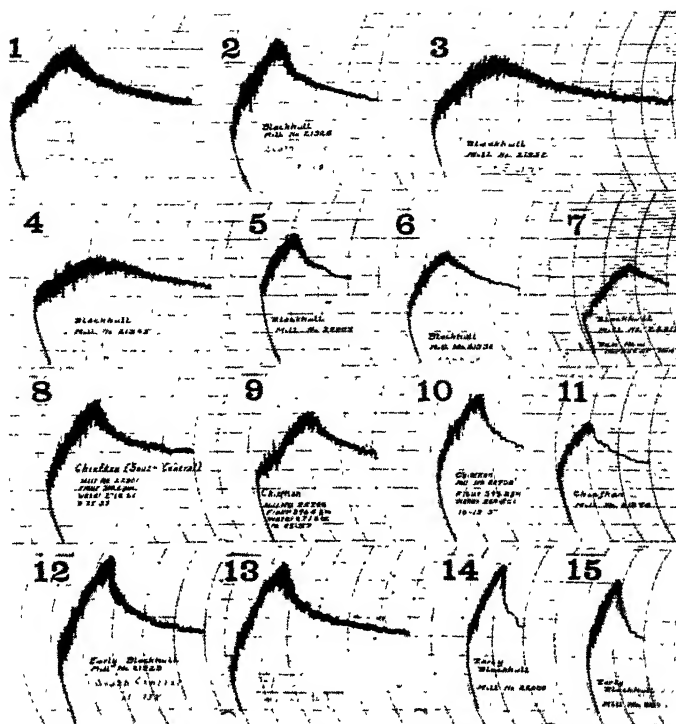


Fig. 6. Blackhull, Early Blackhull, and Chiefkan flours.

No.	Sample	Place grown in Kansas	Year	Test weight Lbs.
<i>Blackhull</i>				
1	20557	S. Central	1935	59.2
2	21928	S. Central	1936	60.7
3	21952	Northeast	1936	61.2
4	21945	Eastern	1936	60.6
5	22003	Hays Exp. Sta. (N. Central)	1936	60.8
6	21936	N. Central	1936	58.6
7	22011	Northwest	1936	61.9
<i>Chiefkan</i>				
8	22901	S. Central	1937	63.1
9	22744	Agronomy Farm, Manhattan (E. Central)	1937	63.6
10	22908	N. Central and N.W.	1937	61.7
11	21974	Agronomy Farm, Manhattan (E. Central)	1936	62.3
<i>Early Blackhull</i>				
12	21929	S. Central	1936	61.8
13	20559	S. Central	1935	60.7
14	22000	Hays Exp. Sta. (N. Central)	1936	63.8
15	21960	Agronomy Farm, Manhattan (E. Central)	1936	63.2

resistance is very short because the increase in mobility is rapid. Curves 11, 12, 14, and 15 have no pronounced turn at the top.

### Width of Curve

Curves 1, 3, and 4 may be characterized as wide or bold. Medium width is found in 2, 5, 6, 7, 11, and 12; narrow in 8, 9, and 10, and very narrow in 14 and 15. Width of curve or boldness is generally obtained from flours which are characterized as very strong or those having a wide tolerance to varying conditions of bread baking. Nar-

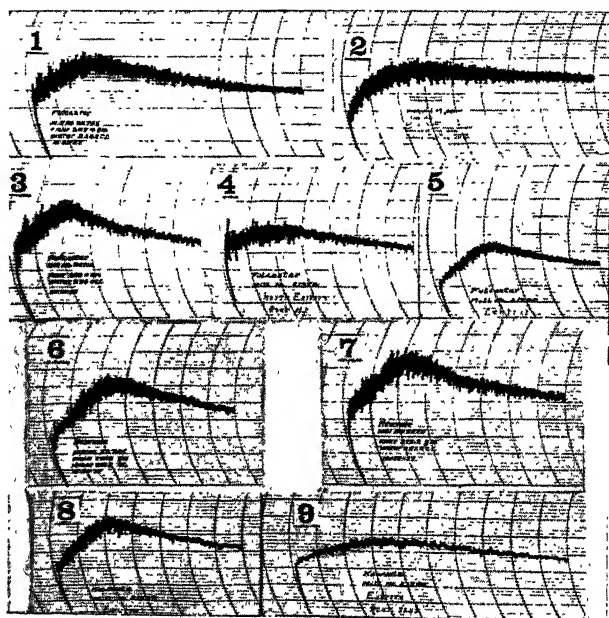


Fig. 7. Fulcaster and Kawvale flours.

No.	Sample	Place grown in Kansas	Year	Test weight Lbs.
<i>Fulcaster</i>				
1	22746	Manhattan (East Central)	1937	61.4
2	20577	Eastern	1935	56.6
3	22912	Central	1937	53.7
4	21954	Northeast	1936	60.4
5	21940	Central	1936	57.7
<i>Kawvale</i>				
6	22745	Manhattan (East Central)	1937	60.3
7	22911	Central	1937	59.9
8	21939	Central	1936	57.4
9	21946	Eastern	1936	58.5

row curves result especially after the peak has been passed, apparently because of a gluten structure which disintegrates more easily under mechanical action. The very narrow curves such as 14 and 15 are exceptional and indicate an abnormal condition.

### Rate of Increase in Mobility

Curves 1, 3, 4, and 11 show very little increase in mobility. The gluten strands apparently continue to slide on each other without breaking. Curves 2, 7, and 12 show a medium rate of increase, while 6 and 8 show a rapid increase and in 9, 10, and 13 the increase in

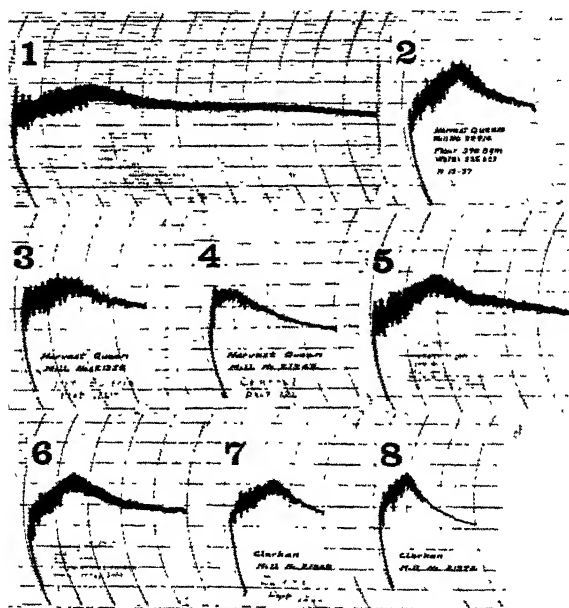


Fig. 8. Harvest Queen and Clarkan flours.

No.	Sample	Place grown in Kansas	Year	Test weight Lbs.
<i>Harvest Queen</i>				
1	20571	Central	1935	55
2	22914	Central	1937	57.5
3	21956	Northeast	1936	59.3
4	21942	Central	1936	57.0
<i>Clarkan</i>				
5	20578	Eastern	1935	59.0
6	20570	Central	1935	58.3
7	21948	Eastern	1936	60.1
8	21972	Manhattan (E. Central)	1936	61.3



mobility is very rapid. This rapid increase in mobility apparently is due either to a breaking of the gluten strands or to more pronounced thixotropic properties. Curves like 14 and 15 have a very small mobility and hence very little or no increase is evident.

### Groups of Curves (Figs. 2 to 9)

The groupings in Figures 2 to 9 were made to bring out how much curves from the same variety may vary not only because of conditions of growth during heading and maturing but also on account of the soil, which is the most potent factor in the protein content. For this reason curves of the same variety were grouped on the same photo-

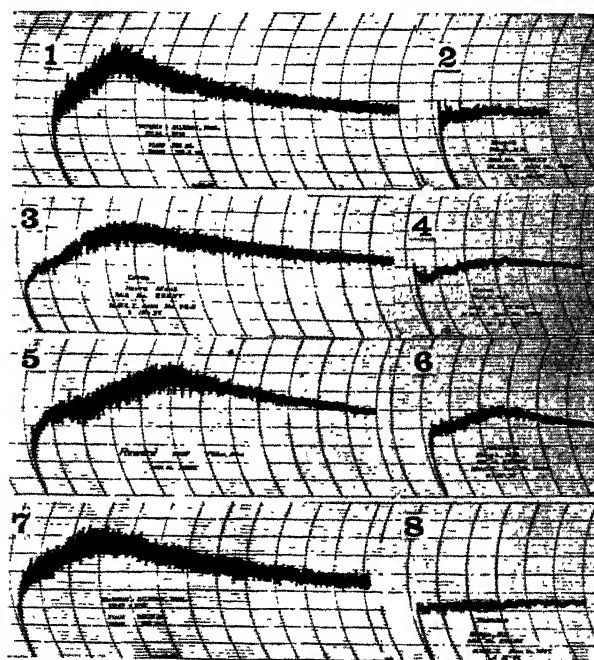


Fig. 9. Spring-wheat flours.

No.	Sample	Variety	Place grown	Year
1	23184	Marquis	Gildford, Mont.	1937
2	22652	Marquis	Fargo, N. D.	1936
3	22657	Ceres	Havre, Mont.	1936
4	22653	Ceres	Fargo, N. D.	1936
5	21345	Reward	Fisher, Minn.	1935
6	22654	Reward	Fargo, N. D.	1936
7	23187	Thatcher	Gildford, Mont.	1937
8	22655	Thatcher	Fargo, N. D.	1936

graph. An attempt is made in the following to place in groups those curves from different varieties which are similar.

The curves in the various figures may be placed in the following groups:

1. Strong bold curves similar to curves 1 to 5 in Figure 1.
2. Moderate short curves similar to curves 6 to 8 in Figure 1.
3. Short and sharp curves similar to curves 9 and 10 in Figure 1.
4. Very rounded or flat curves similar to 11 and 12, Figure 1.
5. Short or rapidly thinning curves similar to 13, Figure 1.
6. Low and thin curves similar to 14 and 15, Figure 1.

#### GROUP 1, STRONG AND BOLD

<i>Fig. No.</i>	<i>Variety</i>	<i>No. of curve</i>
2	Turkey	1, 2, and 3
3	Kanred	1 and 2
4	Tenmarq	1, 2, and 3
5	Cheyenne	1 and 2
7	Fulcaster Kawvale	1, 2, and 3 6 and 7
9	Marquis Ceres Reward Thatcher	1 3 5 7

#### GROUP 2, MODERATELY SHORT

<i>Fig. No.</i>	<i>Variety</i>	<i>No. of curve</i>
2	Turkey	6 and 7
3	Kanred	3, 4, and 6
4	Tenmarq	6
6	Blackhull	1, 3, and 4
7	Kawvale	8
8	Clarkan	5 and 6

#### GROUP 3, SHORT AND SHARP

<i>Fig. No.</i>	<i>Variety</i>	<i>No. of curve</i>
6	Blackhull Chiefkan Early Blackhull	2 and 5 8 and 9 12 and 13
8	Harvest Queen	2

## GROUP 4, VERY ROUNDED OR FLAT

<i>Fig. No.</i>	<i>Variety</i>	<i>No. of curve</i>
2	Turkey	4 and 5
3	Kanred	5
4	Tenmarq	4, 5, and 7
5	Cheyenne	3
7	Fulcaster	4
8	Harvest Queen	1
9	Marquis Reward	2 6

## GROUP 5, SHORT OR RAPIDLY THINNING

<i>Fig. No.</i>	<i>Variety</i>	<i>No. of curve</i>
3	Kanred	8
4	Tenmarq	8 and 9
5	Cheyenne	4
6	Blackhull Chiefkan Early Blackhull	6 and 7 10 and 11 14 and 15
8	Harvest Queen Clarkan	3 and 4 7 and 8

## GROUP 6, LOW AND THIN

<i>Fig. No.</i>	<i>Variety</i>	<i>No. of curve</i>
2	Turkey	8
3	Kanred	7
4	Tenmarq	10 and 11
5	Cheyenne	5
7	Fulcaster Kawvale	5 9
9	Ceres Thatcher	4 8

Not all curves will fit into this classification since some are on the border line between groups, but this grouping shows that flours of various varieties may be similar in their behavior in mixing and likewise that flours of the same variety may be dissimilar. Blackhull, Chiefkan, and Early Blackhull as a group differ markedly from the other varieties although some of their curves find similarities in curves of other varieties.

The outstanding characteristics of the curves from these three wheats are the abrupt turn at the top and the rapid descent, accompanied by rapid narrowing. The two soft wheats, Harvest Queen

and Clarkan, also have as distinguishing characteristics the wideness of the curve at the start and the rapid narrowing during descent.

### Discussion

In considering the usefulness of the curves, it should be emphasized again that these curves were selected to show the widest variability in order to bring out clearly how much curves from the same varieties may differ. These differences are caused by the growth conditions during heading and ripening, and by the protein content. Thus, curves of different varieties should not be judged unless the wheats have been grown under a similar environment and the protein contents are in the same range.

While protein content may influence curve characteristics and must be considered in judging curves, it is not necessarily a determining factor. As a rule low-protein flours will give curves which are flattened, or with tops very rounded and the height much decreased, while high-protein flours tend toward the opposite characteristics.

Critics may say that the curves made on the recording dough mixer have nothing in common with commercial practices, since the mixing goes much beyond the point reached in the commercial bake-shop. The reply to this statement is that the purpose of making these curves is not to imitate commercial practices but to discover physical characteristics of the varieties. In finding the breaking strength of materials it is necessary to go much beyond what it is expected these will have to withstand in practical operation. If the magnitude of the strain is such as to break only the weak, then it will not be known how much of a load the strong can carry. It is very evident that many wheats will yield flours which are stronger than needed in average bread baking. The quality tests should show just how strong these flours are so as to indicate how much strength they may give to the mix which contains the weaker flours. Dough-mixer curves may be used as one of such tests.

Another criticism which may be given on the curves presented is that since the curves can vary so much even in the same variety they have little value in variety testing. It has already been stated that those curves which showed the greatest variability were chosen for these figures. Experience over several years with curves made on varieties has shown that as a rule, probably with 90 per cent of the flours, the curves from any one variety grown under approximately similar conditions will present a pattern of characteristics which is associated with the particular variety tested. Certain varieties like Turkey and Kanred will show strong similarities; likewise will Cheyenne and Tenmarq. These last two will be more like the spring

wheats. Fulcaster and Kawvale, when grown under conditions that favor hard wheat, will give curves which are very similar to those of Turkey. Blackhull, Early Blackhull, and Chiefkan will produce curves which have strong similarities, those of Blackhull having as a rule less sharp tops than the other two. The soft wheats like Harvest Queen and Clarkan in most cases will show quick development of the dough and a rather rapid narrowing of the curve after the peak has been reached.

The use of physical tests is relatively new although, as has been pointed out, the principles have been known to a few people since the beginning of wheat and flour testing. The newness of the use of physical tests should not be held against them nor should other tests be favored simply because they have been used longer and more widely. These physical tests measure certain characteristics, and if they can show wider variation among flours than almost any other test, then the challenge is to learn their interpretation. Much cooperative work has been done to standardize and evaluate the various chemical and baking tests used in cereal laboratories, but little has been done with reference to the physical tests. Only a few cereal chemists have used them and very little collaborative work has been undertaken.

The most serious aspect is that we have thus far no adequate method of evaluating these physical tests. It is a question whether we shall learn the interpretation of these physical tests as long as we use, as a standard, baking-test methods based on formulas and procedures comparable to commercial practices. Such baking tests seem more or less to coddle the weak flours and do not give the strong ones a chance to show all of their possibilities. It may be that what we need is to get away from the idea of a commercially acceptable volume and texture and use a baking procedure the main purpose of which shall be to test the inherent possibilities the same as when engineers measure strength of materials.

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## THE VITAMIN B<sub>1</sub> CONTENT OF WHEAT, FLOUR AND BREAD

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(Read at the Annual Meeting, May 1939)

It has long been known that the production of fine white flour involves the removal of certain otherwise desirable food factors. Prominent among these factors is vitamin B<sub>1</sub> or thiamin, as it is now known. The purpose of the present communication is to present the results of thiamin assays made on several mill streams. This study shows the distribution of thiamin in the various parts of the wheat kernel and also shows what may be expected in flours of various degrees of extraction. Thiamin was determined by our fermentation

method, which has been described elsewhere (Schultz, Atkin, and Frey, 1937, 1938). Substances like wheat, flour, and bread may be analyzed by our method with great ease.

The test takes only three hours and requires but little attention during that time. A number of tests can be run simultaneously. The thiamin does not have to be separated or isolated from the bulk of the sample, and furthermore the test is very sensitive, requiring only 2 to 4 micrograms of thiamin for a satisfactory test. The test is, however, not entirely specific since we have found that 2-methyl-5-ethoxymethyl-6-amino-pyrimidine also gives the test (Schultz, Atkin, and Frey, 1937). This pyrimidine represents a synthetic half of the thiamin molecule and has not yet been found in natural food products. If a product like whole wheat, for instance, is to be studied it is only necessary to have one or two samples analyzed by a reliable animal method. If the assay agrees with the fermentation method, then it may be assumed that interfering substances are absent. This is true in the case of wheat; our results agree with animal-growth tests and are in general agreement with the results of Baker, Wright, and Drummond (1937), who have obtained by the bradycardia method values ranging from 3.6 to 7.8 micrograms per gram on six wheats.

The vitamin B<sub>1</sub> content of various kinds of wheat and the influence of climatic conditions on the vitamin content have not been studied. We believe such an investigation would be well worth while.

Three flour mills have kindly supplied us with samples from their mill streams for the purpose of this study. We are greatly indebted to Mr. Maveety of the National Biscuit Company for one of the samples.

Table I is self-explanatory. The summation in the last column is approximately 10% in excess of the analysis of the original wheat. We do not believe that this discrepancy affects the significance of the data; on the contrary, in view of the possible errors in the sampling of the mill stream and other errors, the agreement is surprisingly good.

TABLE I  
MILL STREAM ANALYSIS—MILL A

	Percent of wheat	Thiamin per gram	Thiamin 1 g. wheat
	%	γ	γ
Wheat	100	6.25*	—
Bran	13	16.0	2.08
Middlings (shorts?)	13	28.0	3.64
Low-grade flour	7.1	4.5	0.319
Bakery flour	64.2	0.85	0.546
Ground Screenings	2.7	6.4	0.173
			6.758*

In Table II we have a somewhat more extensive picture, although the original wheat was unavailable. It is interesting to note that a summation (not in table) of the bran, shorts, and straight flour gives 5.9 gammas per gram for the original wheat, which is a reasonable figure. From the table can be seen the relation between straight, long-patent, and short-patent flour. The summation in the last column in this case shows a very good agreement.

TABLE II  
MILL STREAM ANALYSIS—MILL B

	Percent of wheat	Percent of straight flour	Thiamin per gram	Thiamin 1 g. straight flour
	%	%	$\gamma$	$\gamma$
Bran	13.75	—	13.3	—
Shorts	13.75	—	21.0	—
Straight flour	72.5	100	1.5*	—
Short patent	—	73	0.7	0.51
Fancy clear	—	23	2.7	0.62
Low grade	—	4	10.3	0.41
Long patent	—	90	1.2	—
				1.54*

The mill stream from Mill C provides the most complete picture. Part I indicates the major distribution of the thiamin of the wheat berry. This mill is the only one of the three which milled out the germ. The summation of the wheat fractions shows very good agreement in this case. It is interesting to note that the richest fraction of wheat (the germ) actually contains only 5% of the original thiamin. As the wheat berry contains about  $1\frac{1}{2}\%$  to 2% of germ, it is probable

TABLE III  
MILL STREAM ANALYSIS—MILL C, PART I

	Percent of wheat	Thiamin per gram	Thiamin 1 g. wheat	Percent total thiamin
	%	$\gamma$	$\gamma$	%
Wheat	100	5.70*	—	—
Germ	1	30.0	0.3	5.0
Bran	17	13.2	2.24	37.8
Shorts	10	23.0	2.3	38.8
Straight flour	72	1.5	1.08	18.0
			5.92*	

that the germ accounts for 10% of the thiamin of the wheat berry. In milling practice not all the germ is recovered. Fully 82% of the thiamin finds its way into feeds, and Table IV shows how the remainder of the thiamin is distributed on further refining.



TABLE IV  
MILL STREAM ANALYSIS—MILL C, PART II

	Percent of straight flour	Thiamin per gram	Thiamin 1 g. straight flour	Percent thiamin in straight flour
	%	$\gamma$	$\gamma$	%
Straight	100	1.5*	—	—
Short patent	80	0.7	0.56	41.8
First clear	12	1.8	0.216	16.6
Second clear	7	5.1	0.35	26.6
Red dog	1	21.2	0.212	21.2
First midds	—	0.3	—	—
Third break	—	0.6	—	—
			1.338*	

As may be seen from the last column, more than 50% of the remaining thiamin is removed in the process, which yields a short patent flour from a straight flour. The summation of the fractions which come from the straight flour shows a reasonable agreement.

Included in this table are thiamin assays on two special flours not usually available. The first midds flour, which has the very low thiamin content of 0.3 microgram per gram, represents the best 33% of the short-patent flour and is probably an example of a highly refined flour. The third-break flour (which constitutes about 3½% of the short-patent flour) is also low in thiamin content (0.6 microgram per gram).

The general trend of our data would seem to indicate that the more

### *Distribution of thiamin in wheat milling*

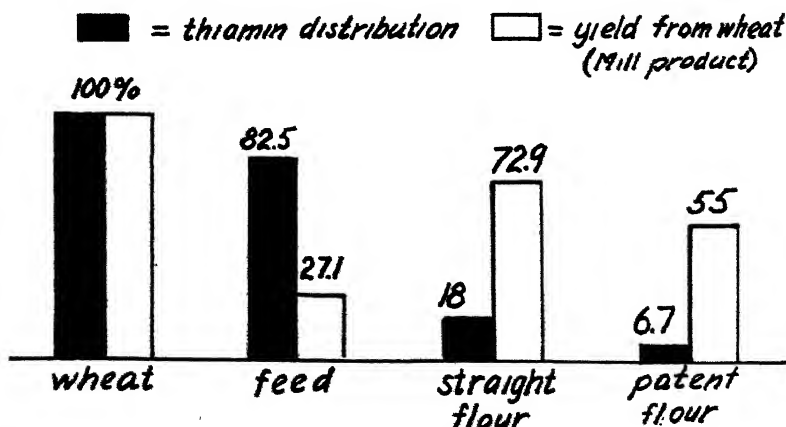


Fig. 1. Distribution of thiamin.

highly refined a flour is the lower will its thiamin content be. Figure 1 summarizes our somewhat limited data.

The bars marked "feed" represent the combined results on bran, shorts, and germ.

What is the relation of these studies to the vitamin content of bread? The following statement of Williams and Spies (1938) from their recent book on vitamin B<sub>1</sub> is of interest:

"In yeast breads there appears to be fairly general agreement that the thiamin content, on an air-dry basis, corresponds very closely to that of the flours used."

In general we find this statement approximately true. The recent advent of a high-vitamin baker's yeast provides, of course, an outstanding exception. The thiamin content of this yeast is so high that it is possible for the baker, using 2% of this yeast based on the flour, to produce a fine white loaf to which has been restored practically all of the thiamin removed in the milling process.

Table V shows thiamin assays made on a series of commercial white breads and whole wheat breads. Included for comparison are values for white bread made with high vitamin yeast.

TABLE V  
THIAMIN IN BREAD

	Thiamin per one- pound loaf
	$\gamma$
White bread	410; 321; 456
Whole wheat bread	1760; 1620
High B, white bread	1650; 1750

Thus it may be seen that the fermentation method applied to milling and baking technology is a useful and reliable tool.

### Summary

The vitamin B<sub>1</sub> content of wheat, flour, and bread has been determined by the fermentation method.

The straight flours analyzed had 1.5 micrograms of thiamin per gram and the short patent 0.7 microgram per gram.

Several mill streams have been analyzed for thiamin content and it has been found that the more highly refined a flour is the less thiamin it is likely to contain.

The thiamin content of air-dried bread roughly corresponds to the thiamin content of the flour except when a special yeast has been used. When a high-vitamin yeast has been employed a white bread may contain as much thiamin as a whole wheat bread.

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## FERMENTATION OF MALTOSE IN THE DOUGH

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(Read at the Annual Meeting, May 1939)

Upon completion of recent studies of maltose fermentation (Schultz and Atkin, 1939) it was thought desirable to extend the investigation to fermentation in the dough. Although flour contains but little maltose compared with about 1% of sucrose, the formation of a dough initiates diastatic activity and the maltose thus produced is the principal sugar involved in the later stages of fermentation.

The rate of fermentation of this complex system may be considered from two angles. First, we can study those factors which may affect fermentation in general and, second, those factors which affect the fermentation of a specific sugar (in this case, maltose). Thiamin, under appropriate conditions, causes a most pronounced increase in the rate of fermentation (Schultz, Atkin, and Frey, 1937, 1938). However, although flour is relatively low in thiamin content, the proportion of flour to yeast is usually so high that the fermentation rate of a dough can seldom be increased by added thiamin. In other words, thiamin is not a limiting factor in the presence of sufficient flour.

The stimulation of fermentation by thiamin is dependent on the presence of ammonia nitrogen or of amino nitrogen in the fermenting medium. Without nitrogen in such form thiamin gives but a slight stimulation and consequently it must be concluded that nitrogen (in proper form) is also a potent stimulator of fermentation. Flour is not unduly rich in this fermentation factor, and as will be shown later it has been found that the fermentation rate of a flour-yeast-maltose-water mixture may be affected by added nitrogen.

Maltose fermentation differs from dextrose fermentation in that it is attended by a considerable induction period which has been described by Blish and Sandstedt (1937). As has been reported elsewhere (Schultz and Atkin, 1939) the addition of a small quantity of dextrose, sucrose, etc., remarkably shortens the induction period. It was also shown that extracts containing maltase, such as a dried yeast extract,

have a similar effect. Since flour contains about 1% of sucrose it is logical to suppose that there will be no induction period of consequence in a flour-yeast-maltose-water system.

Recently Sandstedt and Blish (1938) have attempted to explain differences between flours in the third-hour or "proof time" fermentation rates. The explanation was offered that the flours differed in "activator" content.

If the fermentation rate of such mixtures is examined in the light of the foregoing résumé it will at once be seen that the amino-nitrogen factor must be eliminated before the existence of a specific maltose fermentation factor can be postulated.

### Experimental

The experimental procedure employed was patterned after that used by Sandstedt and Blish with the exception that the gas evolved was measured in gasometers at atmospheric pressure in a manner initially described by Schultz and Landis (1932). To a reaction bottle which contained 20 g. of flour and 0.8 g. of maltose 20 ml. of H<sub>2</sub>O was added in which was suspended 0.6 g. of yeast. The mixture was stirred until uniform and then placed in the machine at 30° C. Readings may be taken at any time but for the present purpose only the gas produced during the third hour was recorded. When other substances were included in the mixture they were weighed directly into the bottles or incorporated in a portion of the water.

Employing a high-grade patent flour, we tested four different forms of nitrogen and Table I gives the results. The stimulations obtained

TABLE I

EFFECT OF ADDED NITROGEN ON THE THIRD-HOUR FERMENTATION RATE OF A YEAST-FLOUR-MALTOSE-WATER MIXTURE MADE WITH A PATENT FLOUR

Addition	Ml. gas produced during third hour
None.....	109
50 mg. ammonium sulphate .....	134
50 mg. carbamide.....	121
50 mg. asparagin.....	140
50 mg. aspartic acid.....	125

make it quite apparent that nitrogen may be responsible for a significant part of the differences between flours. The flour with which these tests were made does not show the high response which may be observed with certain other flours. Table II shows the response obtained with a series of three flours. In the absence of added nitrogen a considerable difference may exist in the third-hour fermentation rate. The addition of ammonia nitrogen makes the rates nearly equal. The first mids flour, however, did not quite equal the rate of the other two

TABLE II  
FERMENTATION RATE OF DIFFERENT TYPES OF FLOUR

Flour	Ml. gas produced during third hour		
	No addition	With added nitrogen	With added nitrogen and thiamin
Straight	110	147	No increase
Short patent	99	143	—
First mids	69	132	+12 ml.

when nitrogen was added. As may be observed in the last column the deficiency was due to lack of thiamin. This correlates with thiamin assays of the three flours, which showed 1.5, 0.7, and 0.3 gamma per gram respectively for the straight, patent, and first mids.

Although the patent flour with which most of our tests were made showed an intermediate response to nitrogen it was thought advisable to make our principal studies on that flour since it more nearly represents an average flour.

It has been known for a long time that amino acids occur in flour. Blish (1918) reported that "normal patent flour contains about 2 mg. of amino-acid nitrogen for every 100 g. of flour, and about three times as much nitrogen in free acid amide form." This is a clear indication that amino nitrogen occurs in flours in amounts which may explain the differences between them. Furthermore, it may be expected that the process of autolysis will sometimes produce further quantities of amino acids by protein breakdown. To see which of the amino acids may be responsible for the increased "proof time" fermentation rate a study of 22 amino acids was made.

In each case the acid was added to the dry flour-maltose mixture and well stirred before addition of the yeast suspension. Of the 22 amino acids tested, 6 showed a stimulating effect, 6 were relatively inactive, and 10 decreased the rate. Table III gives the figures. Since all tests were not made on the same day we have, for purposes of comparison, reduced the control values for the third-hour gas production to 100 and expressed the others on that basis. The quantity of acid added was 50 mg. except for the synthetic acids, of which 100 mg. of the *dl* form was used.

### Discussion

Of the significance of the results with specific amino acids in the amounts employed, little can be said at present. The object of this study was merely to show that these substances and perhaps others of similar activity must be considered as possible factors in the difference in "proof time" rate which has been observed with different flours. In general, our results show that numerous factors enter into maltose

TABLE III

INFLUENCE OF AMINO ACIDS ON THE THIRD-HOUR FERMENTATION RATE OF YEAST-  
FLOUR-MALTOSE-WATER MIXTURES—WITH PATENT FLOUR

Amino acid	Gas production	Amino acid	Gas production
	<i>ml.</i>		<i>ml.</i>
<i>l</i> -asparagine	128	<i>l</i> -tyrosine	96
<i>d</i> -arginine·HCl	121	<i>dl</i> -methionine	95
<i>d</i> -glutamic acid	118	<i>l</i> -histidine·HCl	93
<i>dl</i> -valine	117	tryptophane	93
<i>dl</i> -aspartic acid	115	<i>l</i> -proline	93
<i>dl</i> -lysine·2HCl	111	<i>dl</i> -threonine	92
<i>dl</i> -alanine	104	<i>dl</i> -amino butyric acid	88
<i>l</i> -hydroxyproline	102	<i>l</i> -leucine	87
<i>l</i> -cystine	102	glycine	83
glutathione	102	<i>l</i> -cysteine·HCl	67
<i>dl</i> -phenylalanine	102	<i>dl</i> -nor-leucine	65
Control	100	Control	100

fermentation in the dough but that each factor may (with proper care) be separated and studied individually. The actual fermentation of the dough is then the integration of all factors present.

### Summary

The fermentation rate of maltose may be limited by the absence of several factors: thiamin, dextrose (or sucrose), and amino nitrogen.

In yeast-flour-maltose-water mixtures, thiamin and sucrose are generally present in sufficient quantity and are not limiting factors.

Experiments with nitrogen (in ammonia or amino form) indicate that differences in the third-hour fermentation rate between various flours may be due to differences in their amino-nitrogen content.

The effect of 22 amino acids on the third-hour fermentation rate of a yeast-flour-maltose-water mixture made with a patent flour is described.

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# THE ELECTROMETRIC DETERMINATION OF DIASTATIC POWER OF MALTS<sup>1</sup>

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(Read at the Annual Meeting, May 1939)

The official method of the American Society of Brewing Chemists (1935) for the determination of diastatic power is very time-consuming and is subject to large variations in the values obtained among different laboratories. These variations are probably due to individual differences in technique in carrying out the titration of boiling Fehling's solution. Anderson and Sallans (1937) and Sallans and Anderson (1937) have discussed the shortcomings of this method.

In an attempt to increase the speed with which diastatic power determinations may be completed and to simplify the procedure as much as possible while maintaining reasonable accuracy, the electrometric procedure was tested. Several reagents were tried as well as two electrode systems. The procedure finally adopted is essentially that of Shaffer and Williams (1935) for glucose in biological materials.

## Procedure

The malt infusion and starch solution are prepared according to the official method of the A.S.B.C. (1935). The diastasis is carried out as described by Anderson and Sallans (1937) and a blank is run for each determination as in the official method.

The equipment for measuring the reducing power of the digested starch solution electrometrically consists of the following: A potentiometer capable of measuring 0 to 100 millivolts with an accuracy of one-half millivolt, two bright platinum electrodes made by sealing lengths of platinum wire into glass tubes with provision for connecting to the potentiometer and a salt bridge of agar jel saturated with potassium chloride, a boiling-water bath and a 25° C. water bath, 125-cc. Erlenmeyer flasks, a precision 5-cc. pipette for taking samples of digested starch, and a 10-cc. pipette or a burette for measuring the reagent.

The reagent used is Reagent I of Shaffer and Williams (1935). This consists of

29.6271 g. potassium ferricyanide ( $K_3Fe(CN)_6$ )

4.2233 g. potassium ferrocyanide ( $K_4Fe(CN)_6 \cdot 3H_2O$ )

50 g. sodium carbonate ( $Na_2CO_3$ )

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10 g. sodium bicarbonate ( $\text{NaHCO}_3$ )  
117 g. sodium chloride ( $\text{NaCl}$ )

made up to one liter. Analytical Reagent grade salts are used and the solution need not be standardized.

### Determination of Reducing Power

To determine the reducing power of the digested starch 10 cc. of reagent is transferred to a 125-cc. Erlenmeyer flask and 5 cc. of the starch sample is added. A cork is placed loosely in the flask and the flask then placed in the boiling-water bath for exactly 20 minutes. At the end of this time it is placed in the  $25^\circ$  bath and the temperature readjusted to  $25^\circ$  C. after a few minutes.

The electrode system is set up as follows: The reference electrode consists of a small thin-wall vial containing 10 cc. of ferricyanide reagent and 5 cc. of water in which one platinum electrode and one arm of the salt bridge are immersed. The reference electrode is positive. The other arm of the salt bridge and the second platinum electrode are supported in such a manner that they may be inserted in the neck of the 125-cc. Erlenmeyer flask and immersed in the reaction mixture by raising the flask. A beaker (600 cc.) is filled with water from the  $25^\circ$  bath and the reference electrode immersed in it. Thus both electrodes are at  $25^\circ$  during measurement of the potential of the system.

The corresponding starch blank is run in exactly the same manner. The reading in millivolts of the sample minus the reading of the blank gives the E.M.F. value which is used to obtain the final result in degrees Lintner on the existing moisture basis.

### Experimental

A series of determinations were run on a sample of pure maltose monohydrate and the resulting E.M.F. values were found to have a straight-line relationship to the amount of maltose present.

Two series of malt samples were then selected, one covering the range of 40 to 140 degrees Lintner and the other 130 to 250 degrees Lintner on the existing moisture basis. The diastatic powers of these malts were carefully determined by the official A.S.B.C. procedure and by the electrometric. Both methods were run on the same partially saccharified starch solutions. The series covering the lower range of diastatic power contained 14 malts. That for the higher range contained 16 samples. These data were then used to calculate the equations of the relationship between degrees Lintner and E.M.F. The relationship is a straight-line function for both ranges of diastatic power.



The equation for samples in which 2 cc. of infusion and 100 cc. of starch are used is  $y = 2.734x - 25.49$ , where  $y$  equals degrees Lintner on the existing moisture basis and  $x$  is the E.M.F. For samples in which 1 cc. of infusion and 100 cc. of starch are used  $y = 5.155x - 39.96$ .

If curves are drawn on large sheets of millimeter graph paper using 1 cm. per millivolt and 1 mm. per degree Lintner, the results may be read off directly within 2 to 3 degrees Lintner when the E.M.F.'s are read to one-half millivolt.

### Comparison of Results with Official and Electrometric Methods

In order to compare the two methods eight malts were used. Three malts were in the range below 140 degrees Lintner and consequently were run with 2 cc. of infusion. The other five malts were in the upper range and were run with 1 cc. of infusion. In order to determine the variability of each method, four sets of determinations were run on each malt. The determinations and blanks for each method were carried through in duplicate.

Table IA gives the means of duplicate determinations of reducing power in degrees Lintner and the mean of the four replicate determi-

TABLE I  
COMPARISON OF ELECTROMETRIC AND A.S.B.C. METHODS FOR DETERMINING  
DIASTATIC POWER

Means of Duplicate Determinations of Reducing power in Degrees Lintner and  
Means of the Four Replicate Runs on Each Malt

TABLE IA—LOW-DIASTATIC MALTS WITH 2 CC. INFUSION					
M-1635		M-1643		M-1594	
Elec.	ASBC	Elec.	ASBC	Elec.	ASBC
83.3	88.4	96.0	99.4	136.3	135.2
84.5	85.8	98.5	96.5	141.0	133.8
86.3	86.2	98.5	98.0	140.0	134.5
83.5	84.4	97.0	97.1	138.0	137.5
Mean					
84.38	86.18	97.50	97.74	138.82	135.20

TABLE IB.—HIGH-DIASTATIC MALTS WITH 1 CC. INFUSION									
M-1633		M-1639		M-1592		M-1642		M-1644	
Elec.	ASBC	Elec.	ASBC	Elec.	ASBC	Elec.	ASBC	Elec.	ASBC
156.0	160.7	170.0	171.3	180.0	188.8	172.5	173.2	186.3	190.3
159.5	160.8	161.3	170.2	168.3	176.8	170.0	169.5	178.5	182.2
157.0	156.4	161.8	161.8	179.0	184.3	168.5	176.3	180.5	181.3
157.5	159.4	167.5	163.6	176.0	178.6	169.0	174.3	184.0	188.5
Mean									
157.50	159.32	165.15	166.72	175.82	182.12	170.00	173.32	182.32	185.57

nations for each of the malts run with 2 cc. of infusion using the two methods. Table IB gives the same data for the malts run with 1 cc. of infusion.

Tables II and III give the analysis of variance on the values in degrees Lintner obtained with the two methods using 2 and 1 cc. of infusion.

TABLE II

ANALYSIS OF VARIANCE ON THE DIASTATIC POWER VALUES IN DEGREES LINTNER OBTAINED BY BOTH METHODS WITH 2 CC. INFUSION

Source	Degrees of freedom	Variance	F value	5%	1%
Malts	2	11,679.65	2,369.1	3.55	6.01
Methods	1	3.31	0.67	4.41	8.28
Malts x methods	2	31.04	6.30	3.55	6.01
Exp. error	18	4.93	5.94	2.08	2.85
Dup. error	24	0.83	—	—	—

It will be noted in Table II that the "F value" for interaction of malts and methods is significant but not extremely so. This is probably due to the fact that the two malts with the lower diastatic powers in Table IA are slightly lower when measured by the electrometric method while the third malt runs somewhat high by the electrometric method as compared with the A.S.B.C. procedure. Also the third malt lies very close to the upper limit in diastatic power for which 2 cc. of infusion can be safely used. In Table III the "F value" for the interaction of malts and methods is quite insignificant.

TABLE III

ANALYSIS OF VARIANCE ON THE DIASTATIC POWER VALUES IN DEGREES LINTNER OBTAINED BY BOTH METHODS WITH 1 CC. INFUSION

Source	Degrees of freedom	Variance	F value	5%	1%
Malts	4	1,650.16	55.47	2.69	4.02
Methods	1	212.55	7.14	4.17	7.56
Malts x methods	4	14.15	0.48	2.69	4.02
Exp. error	30	29.75	6.30	1.72	2.20
Dup. error	40	4.72	—	—	—

Also in Tables II and III the error variance between duplicates is much higher for the malts run with 1 cc. of infusion. This is probably due to the increased error in pipetting 1 cc. of infusion although precision-grade pipettes were used and care taken in the experimental procedure. A number of determinations were run with 2 cc. of infusion

and 200 cc. of starch. These data are not included here but there was distinctly less variation between replicates than when 1 cc. was used.

TABLE IV

ANALYSIS OF VARIANCE TO DETERMINE ERROR VARIANCE AND COMPARISON OF PRECISION OF THE TWO METHODS WITH 2 CC. INFUSION

Method	Error variance	F value	5%	1%
Electrometric	5.22	1.13	3.20	5.33
A.S.B.C.	4.63	—	—	—

TABLE V

ANALYSIS OF VARIANCE TO DETERMINE ERROR VARIANCE AND COMPARISON OF PRECISION OF THE TWO METHODS WITH 1 CC. INFUSION

Method	Error variance	F value	5%	1%
Electrometric	25.73	1.31	2.43	3.58
A.S.B.C.	33.78	—	—	—

A comparison of the error variances of the two methods when 1 and 2 cc. of infusion are used is given in Tables IV and V. Where 2 cc. of infusion is used the error variance of the A.S.B.C. method is lower than that of the electrometric method. However, in the series where 1 cc. of infusion is used the electrometric error variance is lower than the A.S.B.C. In both cases the ratios of the error variances give "F values" below the 5% level of significance. This would indicate that there is no important difference in precision between the two methods.

### Summary

The electrometric procedure is simple and requires no great technical skill. Only one reagent is required, which need not be standardized if pure salts are used in its preparation and the ferri- and ferrocyanides accurately weighed out. Any simple potentiometer is satisfactory provided it covers the necessary E.M.F. range. A modified quinhydrone pH meter was used for a large number of routine determinations and while not as accurate as a Leeds and Northrup potentiometer it gave sufficiently accurate results for ordinary routine work. The electrometric procedure is very rapid. One operator can determine the reducing powers of twelve samples including blanks within two hours or less after the enzyme hydrolysis is complete, whereas only four to six samples could be completed in the same time with the A.S.B.C. procedure.

The electrometric method compares favorably with the A.S.B.C. method and is sufficiently precise for use with routine samples.

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## A COMPARISON OF METHODS FOR THE DETERMINATION OF DIASTATIC POWER OF MALTS

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Considerable interest has recently been evidenced in the development of a method for the determination of the diastatic power of malt which from the standpoints of precision and rapidity will prove superior to the Official Method of the American Society of Brewing Chemists (1936). Anderson and Sallans (1937) have applied Blish and Sandstedt's (1933) ferricyanide method for flours to the determination of diastatic power of malts and show that it is much more rapid and somewhat more precise than the A.S.B.C. method. Hildebrand and McClellan (1938) suggested the ceric sulfate titration as a desirable modification of the original Blish and Sandstedt method.

This paper represents a brief report on a comparison of the precision of the A.S.B.C. method, Anderson and Sallans' modification, and the ceric sulfate titration applied to the determination of the diastatic power of malts.

### Methods

Seven malts with different diastatic powers were used and in order to determine the variability within each method, six determinations on each malt were made with each method. The official procedure was used throughout, up to the determination of the reducing power of the digested starch solution, with the exception that two milliliters of the undiluted malt infusion were used in all determinations. For each run a single extraction of the malt and digestion of the starch solution was made, including the blank, and the quantity of reducing materials in the same solutions was determined in duplicate by each

<sup>1</sup> Cooperative investigations between the U. S. Department of Agriculture, Division of Cereal Crops and Diseases, Bureau of Plant Industry, and the University of Wisconsin.

method. The values were then calculated in milligrams of maltose produced by 2 milliliters of infusion. The Lintner value was calculated for the A.S.B.C. method in the usual way and this was multiplied by the ratio of milligrams of maltose by other methods to the milligrams of maltose calculated for the A.S.B.C. method, to obtain values in degrees Lintner for the Anderson and Hildebrand methods. At frequent intervals, the solutions were standardized against a purified dry sample of maltose and the maltose equivalents of the various solutions used in the calculations.

### Presentation and Discussion of Data

Table I contains the means with their standard errors of the six duplicate determinations on each malt and with each method.

TABLE I

MEANS OF SIX DUPLICATE DETERMINATIONS, WITH THEIR STANDARD ERRORS, OF DIASTATIC POWER ON SEVEN MALTS IN MILLIGRAMS OF MALTOSE PRODUCED BY 2 ML. MALT INFUSION AND IN DEGREES LINTNER

Malt No.	Milligrams maltose			Degrees Lintner		
	Anderson	A.S.B.C.	Hildebrand	Anderson	A.S.B.C.	Hildebrand
1	371± 3.2	366± 3.3	398± 5.3	99±0.8	97±1.0	106±1.5
2	510± 4.0	509± 3.4	557± 9.1	136±1.1	136±0.8	148±2.7
3	517± 9.1	523±11.6	577± 3.5	138±2.3	140±2.9	154±1.7
4	580± 7.3	591± 5.0	633± 9.2	154±1.8	158±1.5	169±2.4
5	619± 6.0	619±11.5	672±14.9	165±1.9	165±3.2	179±4.0
6	688±14.2	690±14.2	747±16.8	184±3.7	184±3.9	199±4.3
7	720± 8.1	716±14.5	729±10.5	192±2.4	191±3.7	195±3.0

An analysis of variance was calculated on the data from all three methods and significant F values were obtained for methods and for the interaction of malts with methods. However, when the analysis was applied to data from the A.S.B.C. and Anderson methods only, these values were not significant. Hence the high values for diastatic power obtained with the Hildebrand procedure appeared to be responsible for the significant F values for methods. These high values were consistent for six of the malts, but on the seventh the results agreed well with the other two methods and this probably accounts for the high interaction value for malts and methods.

The variation within the six determinations on each malt, as shown by the standard errors, fluctuates considerably with the different methods and on the different malts, but is only slightly in favor of the Anderson method.

In order to compare the precision of the three methods, analysis of variance was calculated on the values obtained with each method

on all of the malts. The condensed statistics from the calculation using values in degrees Lintner are given in Table II. From this it is seen that the Anderson method has the lowest error variance, being somewhat the most precise of the three. However the comparisons give F values which do not exceed the 5% level of significance, so there is no essential difference in precision between the three methods. The F value obtained in comparing the Anderson and Hildebrand methods equals the 5% point and might become significant if more malts were used.

TABLE II  
COMPARISON OF PRECISION OF THREE METHODS ON ALL OF THE MALTS USING  
VALUES IN DEGREES LINTNER

Method	Error variance	Comparison	F value	5%	1%
Anderson	4.71	Anderson and Hildebrand	1.77	1.77	2.24
A.S.B.C.	7.29	A.S.B.C. and Anderson	1.55	1.77	2.24
Hildebrand	8.35	Hildebrand and A.S.B.C.	1.14	1.77	2.24

Because of the high values and the somewhat erratic results obtained with the Hildebrand method, a further study of this method was undertaken. Comparable results on malt 7 (Table I) were obtained with a new ceric sulfate solution which differed somewhat in concentration from the first solution used. The effect of concentration on the oxidizing power of ceric sulfate solutions has been checked, and no significant effect found within the rather narrow limits used.

In order to determine whether slight errors in the preparation of the solutions might be responsible for the high results, four new stock solutions of ceric sulfate were made up and diluted to 0.0176 normal. Also four new potassium ferricyanide solutions were prepared. These solutions were used in various combinations in determining the diastatic power of seven additional malts. Table III shows the means of two determinations on each malt using the combinations of solutions shown, with the Hildebrand method, and the value obtained with the A.S.B.C. method.

While certain combinations of solutions, for example ferricyanide No. 3 with ceric sulfate No. 4, seem to give consistently higher results than the others, the differences are not great and do not seem to indicate that the high values obtained in the earlier work were due to such a cause. Also in this series of determinations all of the values obtained with the Hildebrand method were appreciably higher than those obtained with the A.S.B.C. method. As in the earlier study the difference between ceric sulfate and A.S.B.C. values varied for different

malts, making the use of a correction factor unsatisfactory. The cause of the high values and erratic behavior of the ceric sulfate method is still unexplained.

TABLE III

MEANS OF TWO DETERMINATIONS OF DIASTATIC POWER IN DEGREES LINTNER ON SEVEN MALTS USING DIFFERENT COMBINATIONS OF REAGENTS FOR THE HILDEBRAND METHOD

Combination of reagents		Malt numbers					Combination of reagents		Malt numbers	
Ferri-cyanide solution no.	Ceric sulfate solution no. <sup>1</sup>	8	9	10	11	12	Ferri-cyanide solution no.	Ceric sulfate solution no. <sup>1</sup>	13	14
3	3	95	110	129	146	187	1	1	150	177
4	3	95	109	129	145	186	2	1	152	180
3	4	98	112	132	148	190	1	2	151	180
4	4	95	110	128	145	183	2	2	157	182
Value with A.S.B.C. method		85	100	115	132	167	A.S.B.C. value		145	172

<sup>1</sup> The ceric sulfate solutions used here were all 0.0176 N.

### Conclusions

On the limited number of malts used in this study, the A.S.B.C. and Anderson methods of determining diastatic power gave values which were very close. The Hildebrand method for some unexplained reason gave erratic results, these being consistently high on six of the malts and agreeing very well on one. Further study failed to find the cause of this disagreement.

There are no significant differences in precision in the three methods, the Anderson method being only slightly more precise than the other two. However, its appreciable advantage in rapidity over the A.S.B.C. method as discussed by Anderson, gives it more value for routine analytical determinations.

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# SOME FACTORS INFLUENCING THE VISCOSITY OF RICE FLOUR SUSPENSIONS

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Pure rice flour is used today as a filler and thickener for many prepared foods. As such its viscosity characteristics in water solution become highly important, especially when the product is a canned food which must be subjected to high pressures and temperatures for sterilization. This investigation was conducted for the purpose of determining the factors responsible for the variation in viscosity of rice flours.

## Material and Methods

Rice flour ground from broken kernels of brewer's rice was employed in these tests. The viscosity apparatus was the Bauer Viscosimeter (Fig. 1). This is a machine having a constant-speed motor turning two paddles immersed in the flour-and-water suspension. The resistance offered to these paddles is registered through a differential on a chart moving at constant speed. The suspension bowl is set in a jacket. By this means, the temperature of the suspension may be controlled from 15° C. to 100° C. by flowing water or steam through the jacket. This instrument is sensitive enough to measure changes of 1% in a glucose solution.

For these tests, one part rice flour to 6.5 parts of water was used. Steam was introduced into the jacket at a constant rate of speed for all tests; the rate of rise in temperature to the gelatinization point was the same in all cases. The viscosity, as demonstrated by the curve, rose as the heat swelled the starch granules. Maximum consistency was attained at 82°–85° C., the gelatinization point. After this, the curve dropped to an average point which it held as long as the temperature was kept constant. Variation in the temperature then brought about changes in the viscosity. Figure 2 shows a typical chart obtained with this apparatus.

A correlation between the high points of the curves and viscosity of rice-flour suspension during and after a canning process was observed. Those flours having the highest maximum points in viscosity as measured by the Bauer instrument had the greatest consistency after the canning process. Therefore, comparison of high points of viscosity curves offered a good method for determining the quality of the flour.



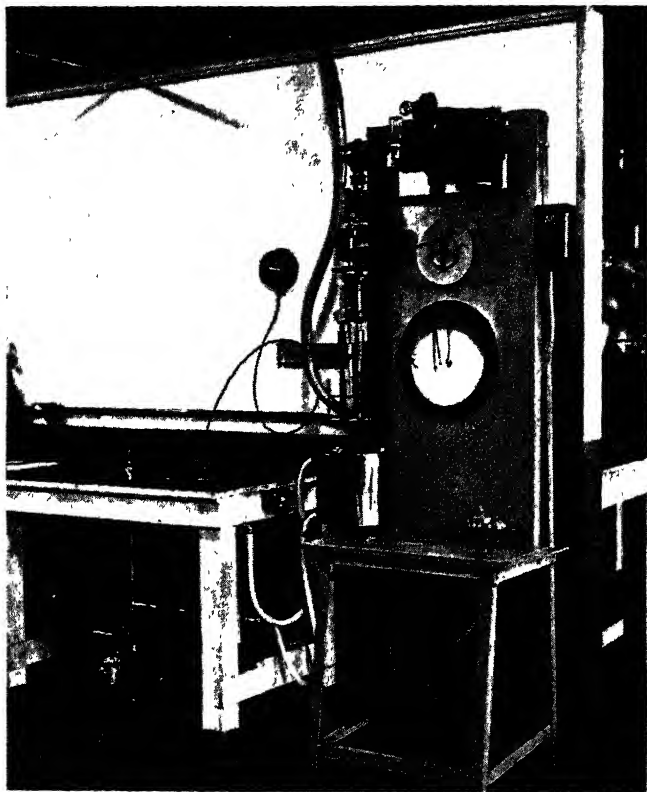


Fig. 1. The Bauer Viscosimeter.

### Effect of Particle Size upon Viscosity

The effect that small or large granules have upon the consistency of rice flour suspensions was first determined.

TABLE I  
RICE FLOUR VISCOSITY AS RELATED TO PARTICLE SIZE

Sample No.	Screen	Viscosity
126	On 60 M.	48
126	Through 60 M.	56
1433-R mill	On 80 M.	66
1433-B mill	On 100 M.	72

These results seem to indicate that the finer granulation of the starch particles permits greater water absorption and results in greater viscosity.

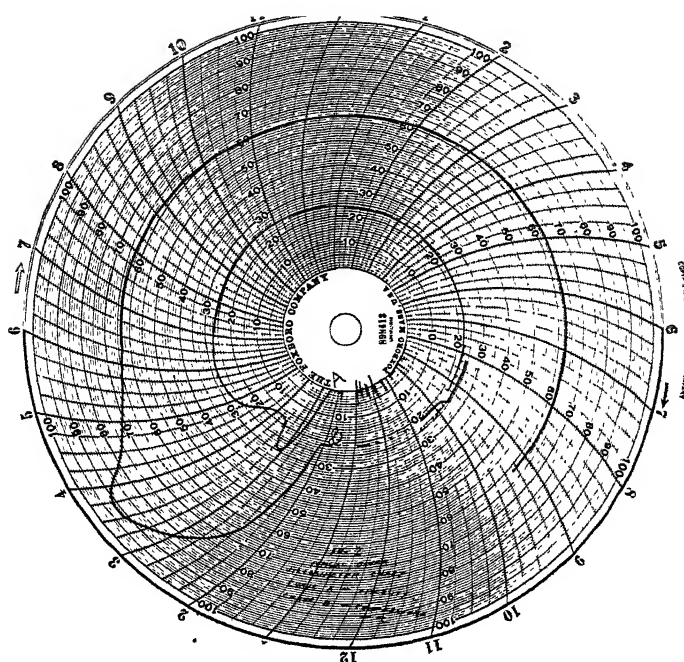


Fig. 2. Typical viscosimeter chart. Curve A, viscosity; Curve B, temperature in Centigrade degrees.

### Effect of pH on Viscosity of Rice Flour

Since it is an accepted fact with authorities in starch chemistry that acid reduces viscosity and alkalis increase the viscosity of starch suspension, pH was determined, electrometrically, on a number of samples in conjunction with viscosity curves.

The figures from Table II seem to indicate that viscosity tends to follow pH. Low pH levels give low viscosities, while in a general

TABLE II  
RELATION OF pH TO VISCOSITY OF RICE FLOUR SUSPENSIONS

Sample No.	Viscosity	pH
1	66	5.5
2	55	6.1
3	57	6.2
4	67	6.3
5	68	6.35
6	72	6.3
7	72	6.35
8	79	6.9
9	82	6.9
10 (pure rice starch)	87	6.95

way high viscosities were obtained at high pH levels. Similarly, it was observed that those flours which had high viscosity curves with a pH close to neutral had the greatest consistency when subjected to a canning process. The critical pH range in which greatest changes in viscosity were noted was 6.4-6.9.

### Factors Influencing pH of Rice Flour

Winton found that rice oil increases in acidity with great rapidity on aging. He also found that this oil contained at times as much as 90% free fatty acids.<sup>1</sup>

Ash and fat were determined on several samples of rice flour by the A.A.C.C. method. The fat was titrated with *n*/10 NaOH using phenolphthalein.

TABLE III

RELATION OF FAT ACIDITY TO pH AND VISCOSITY OF RICE FLOUR

Sample No.	Ash	Fat	Neutralized by 1 g. fat	Viscosity	pH
	%	%	cc. <i>n</i> /10 NaOH		
1	0.896	0.75	26.7	66	5.5
2	0.937	1.25	23.3	55	6.2
6	0.898	1.37	21.8	72	6.3
7	0.561	0.88	22.5	72	6.35
9	1.4	1.46	4.5	82	6.9

Quantity of fat, as such, does not seem to affect the viscosity or the pH. However, the amount of free fatty acid present, as shown by the NaOH neutralization figure, seems to have a bearing on the pH of the flour. Compare Nos. 1 with 9, and 1 with 6.

TABLE IV

CONSTITUENTS OF RICE ASH

(These are average figures.)

P <sub>2</sub> O <sub>5</sub>	SO <sub>3</sub>	SiO <sub>3</sub>	Cl	Na <sub>2</sub> O	K <sub>2</sub> O	CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>
52.1%	0.6%	3.1%	0.09%	5.3%	22.1%	3.4%	11.7%	1.6%

Constituents in the ash also seem to affect the pH. Where the ash percentage is high, the potassium hydroxide and phosphates contained therein can buffer the free fatty acids of the fat. Since these two alkali- and acid-producing compounds are present in varying amounts in different flours, the degree of buffering would also vary. This would give rise to varying degrees and quantities of acidity in the flour.

<sup>1</sup>A. L. Winton and K. B. Winton, *The structure and composition of foods*, Vol. I, p. 146, Wiley and Sons, N. Y., 1932.

It was also found that rice which had been stored longest, even under normal dry conditions, when ground into flour showed the lowest pH and the lowest viscosity. Further investigation showed that those rice lots which were low in ash and fat to begin with, denoting the removal of almost all of the bran, showed the least change in pH on storage. Apparently the low oil content was responsible for this. Storage of two to three months seemed sufficient to begin deterioration of the fat with the liberation of fatty acids.

### Summary

Two factors seem to affect the viscosity of rice flour. They are granule size and pH. The latter seems to be regulated in turn by the amount of free fatty acids present. Rice oil becomes rancid very readily on storage, thus accounting for the free fatty acids. Quantity and composition of the ash apparently are factors in the buffering of these acids. Rice, high in fat when stored for several months' time, has a lowered viscosity, quite possibly due to increase of fatty acids.

## MEASURING FERMENTATION RATE AND GAS LOSSES IN DOUGH<sup>1</sup>

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The device first described by Bailey and Weigley (1922) and substantially redesigned by Bailey and Johnson (1924) for measuring the rate of expansion of, and the CO<sub>2</sub> losses from, a fermenting flour dough has passed through a process of evolution which has resulted in substantial changes since it was last described. Since it has proved singularly useful in our hands and in other laboratories in a variety of connections, it appears desirable to publish the necessary diagrams and descriptions so that it may be made available to those who may find use for it.

In its present and latest form, a heavy copper cylinder 8 cm. in diameter and 20 cm. high, shown at *A* in Figure 1, has been substituted for the glass jar used by Bailey and Johnson (1924). This metal container has several advantages over glass. In addition to the fact that it is not liable to breakage, the copper jar is a better conductor of heat than glass, and hence it, and its contents, reach the temperature of the

<sup>1</sup> Paper No. 1703, Scientific Journal Series, Minnesota Agricultural Experiment Station.

thermostat more promptly. It is enough heavier than glass so that there is less difficulty in keeping it submerged in the water thermostat. Moreover it is so much more sturdy than glass that greater force can be applied in seating the lid *B* against the rubber gasket *C* in closing the jar. If necessary, the copper vessel can be held in a clamp or vice while a wrench is used to grasp the square knob in the center of the lid and thus rotate the latter to hermetically seal the vessel. This is often important in insuring a gas-tight joint at the top of the vessel, without which serious leakage may ensue that vitiates the results of the tests.

It will be observed that there is a shoulder near the top of this copper vessel which tapers to a brazed joint with the threaded brass fitting, that constitutes the actual top of the jar. This fitting, 6 cm. in diameter, is of fairly heavy brass, and is of such dimensions that the ordinary mason jar ring may be used as a gasket (*C*). These rings are readily available at a modest price. The lid is threaded into the top and is, in reality, a standard brass plug that can be purchased from a plumbing supply house.

Dough that is under observation is held in a cylindrical glass beaker shown at *F* in Figure 1. This beaker is 5 cm. in internal diameter by  $11\frac{1}{2}$  cm. high, and is provided with numerous openings about 5 mm. in diameter distributed fairly evenly over the upper 60% of the side-wall surface. The purpose of these openings is to afford opportunity for the  $\text{CO}_2$  which leaks from the dough to move out of the beaker and into the atmosphere of the copper vessel, whence it may be absorbed in strong NaOH solution, used in certain studies to be described later.

In most of the studies of dough with which we have been concerned, a portion equivalent to 40 g. of flour has been found most convenient. This actually involves a quantity of hard wheat flour dough weighing 65 to 70 g., the quantity depending upon the absorption or proportion of water to flour, and the weight of other dough ingredients such as yeast, salt, sugar, shortening, etc. Larger quantities of dough up to the equivalent of 50 g. of flour may be employed in the instance of weaker flours, if such doughs, when fermented, do not overflow the glass beaker. Smaller or larger quantities of dough could doubtless be treated in like manner by an appropriate adaptation of the size of the apparatus.

Two major types of measurements have been made with this device: (1) a direct measurement of the rate of dough expansion and (2) an indirect measurement of the loss of gas from the dough. In the first instance about one-quarter of the space between the glass beaker and the copper vessel up to the level of the lowest row of openings in the side wall of the beaker is filled with 23% sodium chloride. This solu-

tion has an aqueous vapor pressure sufficiently similar to that of an ordinary dough so that the latter does not dry and become crusted over during the period of observation. Moreover it is less likely to absorb  $\text{CO}_2$  from the atmosphere of the cylinder than is distilled water.

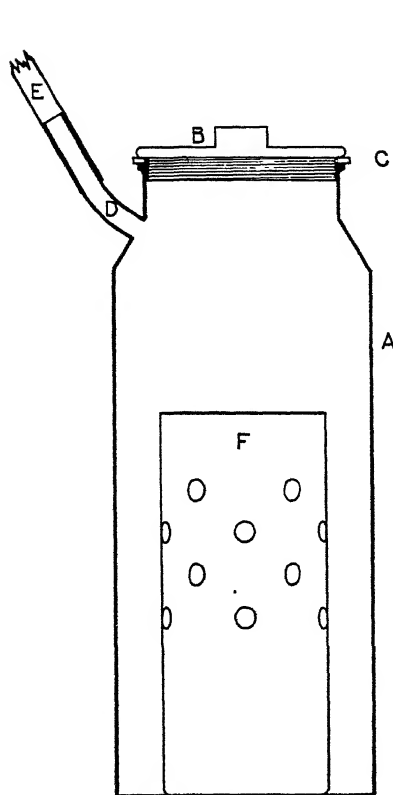


Figure 1.

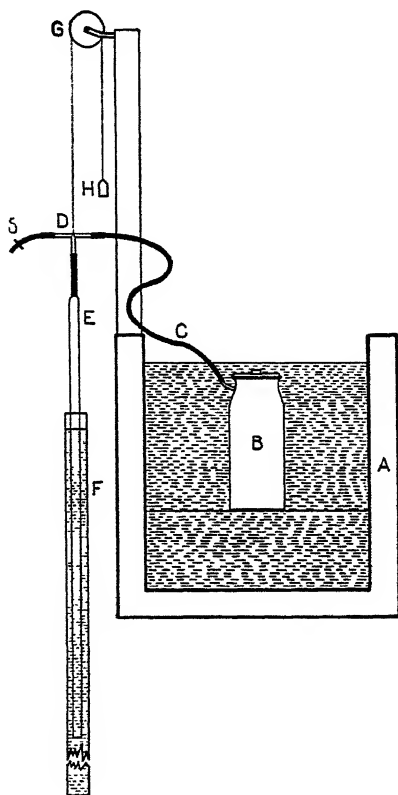


Figure 2.

Fig. 1. Cross section of the copper vessel (A), provided with a threaded brass lid (B) tightly seated against a rubber gasket (C), with a tubulature (D) connected with heavy-walled rubber tubing (E) which discharges the displaced air into a gasometer, and a perforated glass beaker (F) which contains the dough under observation.

Fig. 2. Cross section of the entire gas-measuring system, with a copper lined tank (A) filled with water at constant temperature in which the dough-containing vessel (B) is submerged, heavy-walled rubber tubing (C) connected through a T-tube (D) to the burette (E) of the gasometer which, in turn, is within the larger tube (F) filled with saturated salt solution, a stop-cock or pinch-cock (S) to evacuate the gasometer, a pulley (G) and a counter weight (H) to support the gasometer at the desired levels.

In the second type of measurement mentioned above, an equal quantity of 23% KOH solution is placed in the space indicated. This quickly absorbs the  $\text{CO}_2$  which leaks from the dough. The efficiency can be enhanced by providing a blotting-paper cylinder which fits loosely inside of the copper vessel and thus surrounds the side walls of

the beaker containing the dough but does not cover the top. This blotting-paper cylinder functions as a wick to draw the strong KOH solution closer to the ports in the side of the glass beaker, thus reducing the distance in space through which the  $\text{CO}_2$  must diffuse before encountering a surface moistened with the absorbent. Since blotting paper such as is used in seed germination tests is suitable for this purpose, and is relatively cheap, it is recommended that a fresh cylinder be used for each test.

As a matter of fact, our experience with the device has indicated that  $\text{CO}_2$  is absorbed so promptly in the absence of the paper cylinder that it is probably unnecessary to use it for ordinary work.\* It was indicated above that the volume of  $\text{CO}_2$  which escapes from the dough is actually measured indirectly. Thus the changes in displacement of a dough plus the volume of escaped  $\text{CO}_2$  are recorded in the first vessel, which contains no  $\text{CO}_2$  absorbent. The change in displacement of the dough is observed in another aliquot of the same dough contained in the second vessel which is charged with the  $\text{CO}_2$ -absorbent KOH solution. The difference between these two observations represents the volume of  $\text{CO}_2$  which escapes from the dough in the second vessel. Such losses of  $\text{CO}_2$  from fermenting dough are particularly significant in studies of flour strength, fermentation period, the effect of various flour and dough treatments, and a multitude of other variables involved in flour and dough investigations. These measurements of  $\text{CO}_2$  retention are not afforded by most of the other devices proposed for use in determining the fermentation rate of doughs.

A tubulature shown at *D* in Figure 1 is provided to connect the space within the copper vessel to an external gasometer which measures the changes in dough displacement and gas loss. While various types of gasometer, simple and recording, are available, the one used chiefly by us consists merely of an inverted burette (*E* in Figure 2). It is connected with the tubulature of the copper vessel through a glass or metal T-tube (*D*) and heavy-walled rubber tubing (*C*). A suitable pinch-cock or stop-cock is provided (*S*) on the opposite side of the T-tube for use in adjusting initial pressures in the system, and for venting the contents of the burette in returning it to a zero setting.

The burette in question is partially submerged in a glass tube (*F* in Figure 2) which is about twice as large in diameter as the burette, and a little longer than the latter. This glass tube is closed at its lower end, and is filled nearly to the top with aqueous saturated NaCl solution. The saline solution is used in preference to water because  $\text{CO}_2$  is less soluble in it. As air is displaced from the copper vessel *B* and passes into the gasometer burette *E*, salt solution is forced down-

ward in the latter and out into the glass tube *F*. Periodically the burette *E* can be elevated to level the liquid within and without. A cord attached to the burette passes over the pulley *G* and a counter weight *H* serves to maintain the burette in the position to which it is manually adjusted.

Since the total volume of air displaced from the dough container may, and doubtless will, exceed the capacity of the gasometer burette, the air accumulated in the latter can be vented periodically by opening the pinch-cock at *S*, the burette returned to a zero setting, and the system again sealed at *S*. The routine here described obviously serves to maintain the air in the dough container at, or very close to, atmospheric pressure, which is the level of pressure under which it is normally fermented in practice. While an automatic gasometer has certain advantages, such devices are much more complicated and in general are more liable to the leakage which may proceed unsuspected and thus give rise to incorrect records. Moreover they are much more expensive, especially when provided with kymographs. When the volume of work is small, such automatic units may be justifiable on the score of conserving the operator's time. With a large volume of work involving a dozen or more doughs the operator finds his time is practically occupied with this one operation, however, and under such circumstances the necessary attention to the periodic adjustment and reading of the burettes can be given incidentally to the general program of fermentation studies. Moreover any leakage or other faults in operation of the device may be promptly detected under this system of manual control with a consequent reduction in the proportion of faulty determinations.

Since the dough and the air in the copper dough-container must be maintained at a known and controlled temperature throughout the period of observation, provision is made for immersing this copper vessel in a water thermostat. While an air thermostat might suffice, it is usually easier to maintain a constant temperature in a water bath. In this laboratory the water thermostat consists of a long narrow cyprus box shown at *A* in Figure 2 in cross section. It is long enough to accommodate 12 copper vessels. The box is lined with copper, and a false bottom of this metal is provided as shown in the diagram, which is about one-third of the distance between the bottom and top of the tank. A circulating pump is provided which drives the water briskly along the bottom of the bath from right to left. At the left-hand end a slot is provided in the false bottom, not shown in the diagram, through which the circulating water may rise from the lower to the upper compartment. Thence it continues its movement back through the



upper compartment from left to right, returning to the pump where it started circulating. This vigorous circulation is essential to the maintenance of a uniform temperature in all parts of the thermostat.

Close to the pump a sensitive thermoregulator is also submerged in the water, which is connected through suitable relays to a battery of immersion heaters that supply the heat lost by the water while traversing the bath. Any one of several makes or designs of thermoregulators and immersion heaters are doubtless adequate for this system of control. While the description of control and of water circulation may sound as though it is complicated, as a matter of fact it is relatively simple, and can be assembled at moderate cost from standard equipment by anyone who is at all ingenious.

Emphasis should be laid upon the necessity of keeping the copper dough-container completely submerged during all such observations in order to insure that the temperature of its contents is maintained constant. If the vessel is too buoyant to stand steadily on the false bottom of the water-bath, lead weights should be attached to hold it down. Also it should not be jarred or disturbed during the progress of dough-fermentation tests, since the dough may be caused to fall if disturbed during the later stages of fermentation.

Technicians and cereal chemists who are concerned with fermenting doughs will discern many applications for such a device. It is useful in following the changes in fermentation rate, and fermentation tolerance as induced by the inclusion of different sugars in varying amounts, diastatic enzymes, flour and dough "improvers," and other treatments which are reflected in fermentation behavior. The doughs may be studied at any state, either direct from the mixing machine or after fermentation of more or less duration. Doughs may be removed from the vessel, "punched," and returned for additional periods which simulate commercial or laboratory dough fermentation in preparation for baking. These are only a few of the numerous applications made of it in recent years.

### Summary

A simple and relatively inexpensive device, assembled largely from ordinary laboratory equipment, is described which makes possible the measurement of (a) fermentation rate in a yeast-leavened dough, and (b) the loss of  $\text{CO}_2$  from fermenting doughs. A special copper vessel to hold the dough is desirable, which is heavy, rugged, easy to seal tightly, and a good thermal conductor. While the device here described is designed for manual operation, it is possible to add various automatic features. All operations can be conducted at atmospheric pressure.

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## THE EFFECT OF SMALL QUANTITIES OF MALTED OAT FLOUR ON THE KEEPING QUALITY OF WHEAT FLOUR

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The keeping quality of wheat flour has been the subject of many investigations (Swanson, Willard, and Fitz, 1915; Saunders, Nichols, and Cowen, 1921; Whitcomb, Day, and Blish, 1921; and Fisher, Halton, and Carter, 1937); however, it still continues to be a topic of major interest. The fact has been definitely established that a good grade of hard-wheat flour manufactured from wheat that is in good condition will retain its baking quality for a period of time that can be measured in terms of years. In fact there is generally an improvement in baking quality during the first six to ten months of storage. In contrast to the long periods of time during which hard-wheat flour may be stored without deterioration, it is a recognized fact that soft-wheat flours, flours containing self-rising ingredients, and "clear" grade have more limited keeping properties even under good storage conditions.

The question of why the baking value of a clear grade flour will decline as the storage period increases, while the patent flour from which the clear was removed continues to improve with age, is a problem worthy of consideration. As pointed out by Sullivan, Near, and Foley (1936), it is a well known fact that the presence of increasing percentages of wheat germ in flour harms the baking quality in proportion to the quantity present. Clear flours contain a much higher percentage of germ stock than do patent flours. It was generally believed that the fat constituents of the germ were the cause of the harmful effects on baking quality even before there was much experimental evidence to substantiate the belief. It has now been shown by Sullivan, Near, and Foley (1936) that the fat from fresh germ is not deleterious to flour quality and that it is only after unsaturated fatty acids and their subsequent oxidation products

develop during storage that the injurious properties are encountered. Elaborate and complicated theories (Moreau and Dufraisie, 1926) have been built up to explain the effect which certain substances have on the oxidative and hydrolytic reactions of the lipids. Irrespective of the theory involved, the fact is well established that oat flour has antioxidant properties. See papers by Musher (1935) and Conn and Asnis (1937).

The present study was instigated to determine whether additions of malted oat flour to a hard-wheat clear flour could be made to serve effectively the dual purpose of enhancing the diastatic activity of the clear flour and simultaneously reducing the deleterious effects of storage oxidation.

Flour millers have for many years adjusted and standardized the diastatic activity of their flours. This is a necessary control measure because of the unpredictable changes in the diastatic activity of wheat due to causes such as varietal differences, environmental conditions during the growing period, and harvesting and storage conditions. Regulation of the diastatic activity of flour can most satisfactorily be accomplished by adding a small amount of malted wheat flour. The entire subject of the theory and practice in the diastatic treatment of flours has recently been reviewed by Epstein and Schreier (1938).

### Description of Experiments

In order to determine experimentally the value of additions of malted oat flour to wheat flour, a strong hard spring wheat clear grade was selected for this investigation. The clear flour was unbleached and undiastated and had the following analysis on a 15% moisture basis: protein 14.7%, ash 0.64%, diastatic activity 187 mg. of maltose. Since freshly milled flour is not normally at the peak of its baking performance the clear was stored in a flour warehouse for three weeks. Following this storage period, the flour was subjected to baking tests as indicated in Table I, test No. 1. The flour behaved in a normal manner during the baking test and showed the usual lack of sustained gas production that is characteristic of undiastated flours, during an extended fermentation period. One portion of this flour was intimately mixed with malted wheat flour and another portion similarly treated with malted oat flour. The two flours thus treated were adjusted to the same approximate diastatic activity (244 mg. maltose) and gassing power as indicated in Figure 1. The diastatic activities were determined by the Blish and Sandstedt (1933) method and the gassing powers were determined by the method of Sandstedt and Blish (1934).

The rates of gas production over a six-hour period for the two flours were remarkably similar. Further evidence that malted oat flour does not differ markedly from malted wheat flour in its action as a diastatic supplement is shown by the baking tests. The bakings conducted immediately following the additions of the two types of malt to the wheat flour were almost identical, as is indicated in Table I, test No. 2. Additional baking tests were conducted at intervals for a period of eight months. Relatively highly diastated flours were purposely prepared in order to exaggerate any differences which the two types of malt supplements might be expected to cause.

Because of the importance of the baking test in this experiment it was not deemed expedient to rely on a single baking procedure. The policy adopted was to use the method of the American Association of Cereal Chemists (1935) with the following variations designed to detect changes in a flour's ability to withstand both extended mixing and fermentation periods.

1. Three-minute mixing time; 3-hour fermentation period.
2. Three-minute mixing time; 4-hour fermentation period.
3. Three-minute mixing time; 5-hour fermentation period.
4. Four-minute mixing time; 3-hour fermentation period.

All bakes were made in duplicate and the bread was scored the following day. Numerical values were assigned to such internal and external characteristics as volume, symmetry, bloom, break, color, grain, and texture. Platt (1931, 1933) has adequately pointed out the difficulties and pitfalls encountered in grading foods, particularly bread; nevertheless when the limitations are understood, scoring continues to be the best criterion of the relative quality of two products. In Table I are recorded the detailed results of the effect of storage, in a warm room, on the baking properties of the two flours under consideration. The data recorded under the heading "Average loaf volume and bread score" in Table I indicate in concise form the changes taking place in the baking value of the two flours, as tested by the four baking procedures, over an extended storage period. There is thus incorporated in the composite values the effect of age on both the mixing and fermentation tolerances of the doughs.

### Discussion of Results

The importance of adequate gassing power for the production of quality bread has long been recognized. Baking tests and a record of the gas production over a six-hour period (Fig. 1) indicate that malted oat flour is satisfactory as a diastatic supplement to wheat flour. An inspection of Table I indicates that at the start of the experiment the

unsupplemented clear was improved in baking properties to an almost equal extent by the addition of either wheat or oat malt flour.

During storage both flours consistently declined in baking value. There was a gradual reduction in loaf volume and bread score. The reduction in bread score was the result of decreases in loaf volume, inferior loaf appearance, and less desirable grain. The only grading item which tended to increase the bread score was an improvement in

TABLE I

THE EFFECT OF STORAGE ON THE BAKING PROPERTIES OF A CLEAR FLOUR CONTAINING WHEAT MALT CONTRASTED WITH THE SAME FLOUR CONTAINING OAT MALT SUPPLEMENTS

Test No.	Flour storage period	Type of malt supplement	Baking procedure								Average loaf volume and bread score	
			3-hr. fermentation, 4-min. mix		3-hr. fermentation, 3-min. mix		4-hr. fermentation, 3-min. mix		5-hr. fermentation, 3-min. mix			
			Vol.	Score	Vol.	Score	Vol.	Score	Vol.	Score	Vol.	Score
1	Days 21	None	cc. 630	% 91	cc. 620	% 92	cc. 520	% 88	cc. 380	% 76	cc. 538	% 86.7
2	25	Wheat Oat	690 660	92 93	680 680	93 93	585 600	89 90	455 480	83 83	603 605	89.2 89.9
3	91	Wheat Oat	685 610	92 90	645 630	90 90	580 590	87 87	440 470	79 80	588 574	87.7 86.8
4	122	Wheat Oat	637 668	92 92	602 657	89 89	475 515	79 80	410 425	75 74	530 565	83.7 83.7
5	154	Wheat Oat	610 646	86 87	625 692	91 92	553 590	86 85	450 475	78 79	558 602	85.3 85.9
6	185	Wheat Oat	560 590	87 88	650 690	93 94	485 555	81 85	445 450	79 79	536 570	85.0 86.4
7	248	Wheat Oat	518 580	85 85	635 675	93 94	518 582	84 86	438 445	76 76	528 573	84.5 85.3

crumb color. For the first four months there was only a slight difference between the baking character of the two flours and therefore no appreciable evidence that oat flour had made a contribution toward enhancing the keeping quality of the product. However, beyond a four months' storage period it appeared that oat-flour supplements had made a slight contribution toward retarding deterioration. Both the loaf volumes and bread scores of the clear containing malted oat flour had increased, as evident in Table I.

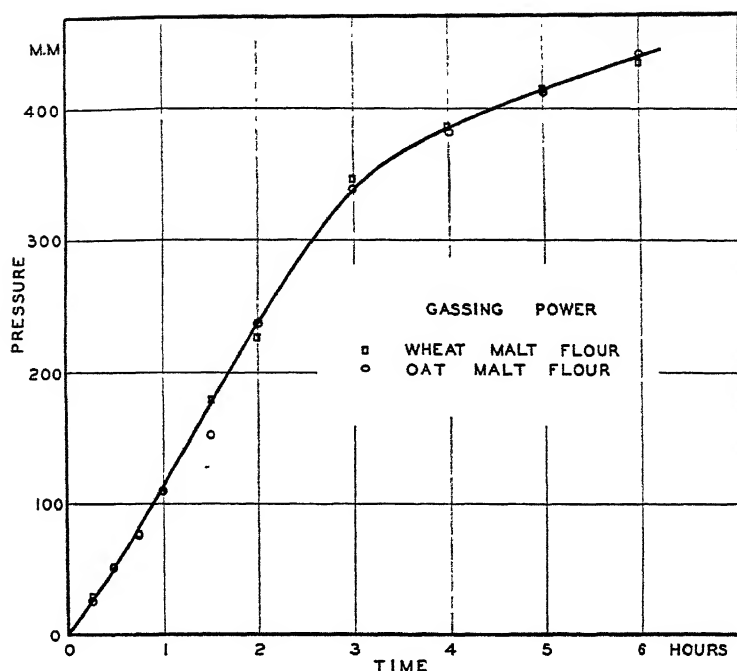


Fig. 1. Gassing power values of the two flours.

### Conclusions

Malted oat flour can be successfully utilized to enhance the diastatic activity of wheat flour.

When two portions of a clear-grade flour were adjusted to the same diastatic activity by additions of malted wheat flour to one portion and malted oat flour to the other, it was found that the baking value of the two flours remained practically identical for several months.

Following several months of storage it appears that malted oat flour exerts some influence toward decreasing the rate of deterioration of flour.

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## VARIATION IN THE BAKING QUALITY OF WHEAT DURING STORAGE

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At the beginning of any wheat crop movement, urgent demand requires that the cereal chemist immediately determine the baking quality of this new supply. The opinions formulated early in the crop movement almost invariably receive extensive publicity in trade journals and frequently set, for the entire season, the popular conception of the crop's quality. It is important therefore, since wheat is a changing commodity, to consider the state of maturity of the grain and the effect which this may have on the estimation of baking quality.

The effect of storage on the baking quality of wheat has been studied by many investigators. This subject has been adequately reviewed and extensive literature citations have been published in books by Bailey (1925) and Swanson (1938); the results indicate rather conclusively that storage enhances the baking quality of wheat. From these findings it is apparent that freshly harvested wheat, which is necessarily used at the beginning of the harvest period to determine the baking quality of the new crop, is at a low point in its baking-quality

cycle. The validity of the experimental baking test as a means of predicting the true potential baking quality of wheat at this period may be questionable. The present investigation was undertaken to determine to what extent the baking properties of wheat are modified during the first few months after storage and also to ascertain whether the viability of the wheat is related to this change.

Practically all seeds require a certain amount of aging before they will germinate properly. The wheat seed is no exception to this rule. One of the most evident signs of the physical and chemical changes taking place in wheat, after cutting, is the gradual increase in viability. Atkinson and Jahnke (1918) and Swanson (1931) have shown that the modification in new wheat which increases its viability reaches a maximum in the comparatively short time of approximately two months.

The inferior baking quality of dead wheat has been shown by Swanson (1926). Since zero viability rather than appearance is the criterion of the extent of damage to the baking properties of dead wheat, one would expect a relationship between the viability of wheat and its baking performance. However, it should be recognized that low viability caused by old age or damage is probably fundamentally different from the low viability of newly harvested wheat.

### Description of Material

Nine samples of wheat of known harvesting date were obtained for this study. The wide geographical area from which the samples were obtained should reduce to a minimum the possibility of dealing with a preponderance of abnormal wheat resulting from the influence of unusual climate, variety, or harvest conditions. The data presented in Table I indicate the origin, harvest date, classification, test weight, and chemical analysis of the samples. It is evident that wide variations of wheat properties are represented in Table I. Some of the samples had to be shipped long distances, and therefore the first milling and baking tests were conducted when the wheat was approximately two weeks old. The investigation of Fitz (1910) indicated that wheat improves in baking quality during the first few days of storage. It is seldom practical to obtain grain immediately after it is threshed; consequently the majority of baking tests of new wheat are conducted on samples which are at least a week old.

The initial baking tests reported in Table III were conducted, it is believed, on wheat of about the usual age of commercial grain at the time when cereal chemists are formulating their opinion of the baking quality of the new crop.

The wheat samples on arrival were placed in burlap sacks and stored in an unheated warehouse. Portions were removed from the sacks for



TABLE I  
DESCRIPTION AND CHEMICAL COMPOSITION OF THE WHEAT SAMPLES

Sample No.	Where grown	Harvest date	Class	Moisture	Protein	Ash	Test weight	Germination	
								Two wks. after harvest	Six mos. after harvest
				%	%	%	Lbs.	%	%
1	Kansas	June 20	HRW	13.1	12.0	1.74	60.3	47	92
2	Ohio	July 8	SRW	13.4	10.3	1.85	60.5	71	98
3	Nebr.	July 6	HRW	10.1	13.4	1.88	60.1	72	83
4	Kansas	July 5	HRW	12.5	12.6	1.76	58.0	51	93
5	S. Dak.	July 22	HRS	13.1	14.5	1.94	57.5	60	97
6	N. Dak.	July 27	HRS	12.0	12.9	1.80	61.1	79	91
7	Wash.	Aug. 4	HW	9.1	14.4	1.89	60.5	97	98
8	Minn.	July 25	HRS	12.0	15.6	2.00	53.0	88	82
9	Mont.	Sept. 19	HRS	9.3	12.1	1.74	59.8	95	98

TABLE II  
AVERAGE ANALYSES OF THE NINE FLOURS OBTAINED FROM MILLING THE WHEAT SAMPLES AFTER VARIOUS STORAGE PERIODS

Storage period	Germination	Flour yield	Protein	Ash	Viscosity	Dia-static activity	Gassing power
Days	%	%	%	%	°McM.	mgs.	mm.
15	73	60.9	11.6	0.42	118	129	223
34	83	60.3	11.5	0.42	120	130	216
106	96	61.2	11.5	0.43	115	117	208
180	93	62.2	11.5	0.44	124	121	224

germination, milling, and baking tests, at the time periods indicated in Table II.

### Experimental

In Table II is recorded the average germination test of the nine wheat samples and the average change which took place in the analyses of the samples of flour milled from these wheats periodically during a six months' storage period.

The viability of the seeds was tested by making germination determinations. The first germination test, made when the samples were approximately fifteen days old, varied from a low value of 47% germination to a high value of 97%. The average for all samples was 73%. After a month's storage the average germination for all samples had risen to 83%, and the maximum value was reached approximately three months after harvesting; also the best baking results were ob-

tained at this period of maximum viability. This point will be elaborated later.

The wheat samples were milled on the experimental mill described by Libby and Shellenberger (1938). The mill was operated under the temperature and humidity conditions prevailing in a commercial flour mill. The milling procedure paralleled closely that described by Markley (1936). Each of the nine samples was milled four times during the six months' storage period. Every effort was made to reproduce, as closely as possible, the same milling procedure each time the samples were remilled.

The operation of an experimental mill for wheat testing always adds an additional variable. Geddes, Bergsteinnson, and Hadley (1933) have shown that the differences in flour characteristics due to experimental milling are not without their influence on the baking test.

The information recorded in Table II indicates that the ash content of the flour is the only determination that shows a consistent trend. Although it is true that the utilization of the readily convertible carbohydrates of the wheat kernel, by the process of respiration, tends to leave a higher percentage of ash in wheat after prolonged storage, nevertheless no such increase as 0.02% can be accounted for on this basis during a storage period of only six months. The indicated increase in ash content of the flour is probably the result of variations in the operation of the experimental mill as indicated by the flour yield data. The protein, viscosity, diastatic activity, and gassing-power values remained relatively constant.

Because of the importance of the baking test in this experiment, it was not deemed expedient to rely on a single baking procedure. The policy adopted was to use the basic A.A.C.C. procedure (Blish, 1928) plus the following three supplementary methods:

- (1) The addition of  $\frac{1}{4}$  of 1% malt flour,
- (2) The addition of  $\frac{1}{4}$  of 1% malt flour plus 1 mg. of potassium bromate,
- (3) The addition of  $\frac{1}{4}$  of 1% malt flour plus mechanical modification of the dough.

Each sample was baked by all four methods on two successive days, and the average loaf volume and bread score of the two bakes were recorded. Thus the values for loaf volume and bread score recorded in Table III represent the average of 36 test loaves, and each flour sample was baked and scored 144 times during the course of this investigation. Bread scores were determined by grading the bread the day following baking. All the important internal and external characteristics of the loaves were considered during the scoring process.

TABLE III

AVERAGE LOAF VOLUME AND BREAD SCORE OF THE NINE SAMPLES OF WHEAT AS DETERMINED BY FOUR BAKING PROCEDURES DURING A SIX MONTHS STORAGE PERIOD

Storage-period	Ab-sorption, 15% m.b.	Baking procedure								Average for all bakes	
		A.A.C.C.		Malt flour supplement		Malt plus 1 mg. of KBrO <sub>4</sub>		Malt plus extended mixing <sup>1</sup>			
Days	%	Vol.	Score	Vol.	Score	Vol.	Score	Vol.	Score	Vol.	Score
15	57.3	485	81.6	551	86.8	636	91.4	600	81.3	567	85.3
34	57.7	496	82.9	583	89.5	649	92.1	597	81.8	581	86.5
106	58.1	460	82.9	576	89.4	638	92.4	576	90.4	564	88.8
180	57.9	458	81.0	566	88.6	634	92.0	601	91.4	568	88.4

<sup>1</sup> Mixing time 4 minutes in a Hobart-Swanson mixer.

### Discussion of Results

No conclusions were attempted based on the study of an individual wheat sample, only the average changes which all samples underwent being considered in this report. It is believed that the data and findings herein presented afford a reliable indication of the changes which wheat undergoes during normal storage.

The mean germination for all samples two weeks after cutting was 73%. Two of the wheat samples, because of shipping conditions, arrived with only slightly over 9% moisture; and these two samples had unusually high germination capacity. The fact that the careful drying of grain enhances its germination energy has been recognized and utilized in the malting industry (Kropff, 1927), and therefore the desiccation the wheat samples sustained in this case can be considered a contributing cause for the high average value of 73% germination 15 days after cutting. The increase in viability as the storage period increased indicates that the samples were normal in this respect.

In Table III is recorded the mean absorption, loaf volume, and bread score, as determined by the four baking methods, of all nine samples, for each of the four storage periods. This information is presented graphically in Figure 1. A portion of the area under the loaf-volume curve is sectioned to set it apart from the three upper curves because the ordinates represent different units. A casual inspection of the loaf-volume curve might suggest that a very significant change in the volume had occurred, but actually the mean values for all bakes differed by only 17 cubic centimeters. The average of all baking data indicate that the loaf volumes remained remarkably

constant during the six months' period; however, loaf volume is only one of the many characteristics of bread which should be considered when evaluating baking quality. When all the important internal and external characteristics of the bread are considered it becomes evident that as the wheat matured there was an improvement in the baking quality. Considering the bread score as the criterion of baking quality, it is evident that the best bread was produced when the wheat was approximately three and one-half months old. Also this storage period coincides with the maximum dough absorption and greatest germination capacity. The bread score values, however, indicate

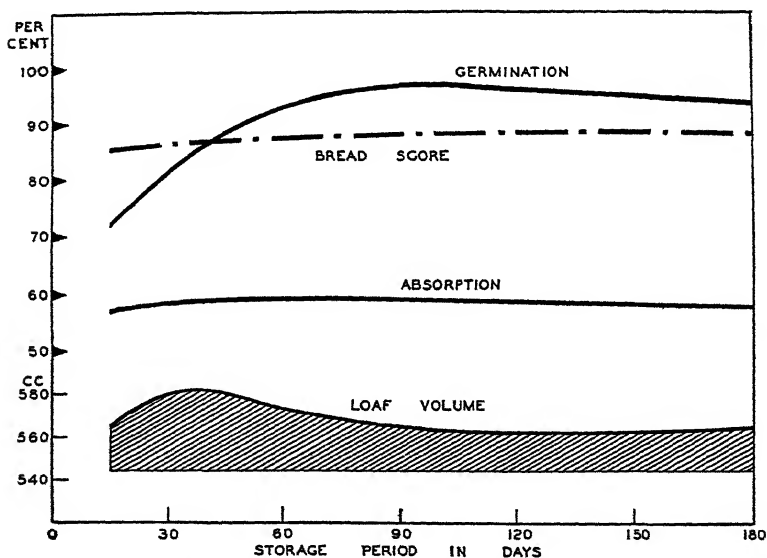


Fig. 1. Effect of storage on germination, absorption, loaf volume, and bread score of wheat samples.

that the improvement noted in baking value is not particularly impressive. In fact, the data indicate that a surprisingly true picture of the potential baking value of wheat can be obtained almost immediately after harvest, assuming, of course, that the grain has been allowed to mature properly before cutting.

### Conclusions

The baking quality of wheat is improved by storage after harvest, but the betterment observed during this investigation was not particularly impressive. This conclusion is based on the study of four classes of wheat, obtained from eight states, during the 1938 harvest.

The experimental baking test, applied to new wheat, provides a reliable indication of the potential baking quality of the crop.

There appears to be a direct relationship between the viability of wheat and its baking quality.

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## EFFECT OF TEMPERATURE ON DOUGH PROPERTIES, II

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(Read at the Annual Meeting, May 1939)

In the ordinary baking of bread a crust is quickly formed which interferes with the study of the properties of the interior dough during baking. The changes in the properties of dough, while baking, progress in sequence from the exterior to the interior; thus no two zones are under the same condition of change at the same time. In order to obviate these difficulties a method of heating bread electrically in which no crust is formed, and in which the entire mass rises in temperature uniformly and under controlled conditions, was developed by Baker (1939).

Figure 1 is a drawing of an improved baking pan showing the relation of the various parts to each other. Believing that the proper-

ties of the dough determine the character of the bread, we have used various means to test the dough while being heated, so that its changes could be detected and studied. The rate of rise becomes a measure of volume because the pan is straight-sided and thus restrains the dough between the electrode walls throughout its oven spring. In order to obtain a uniform heat input in the dough, a constant wattage is applied. The voltage required to keep this wattage constant varies with the changes in resistance of the dough and therefore is inversely indicative of the changes in conductivity of the dough while baking. The passage of a weight through the dough mass while heating gives evidence of

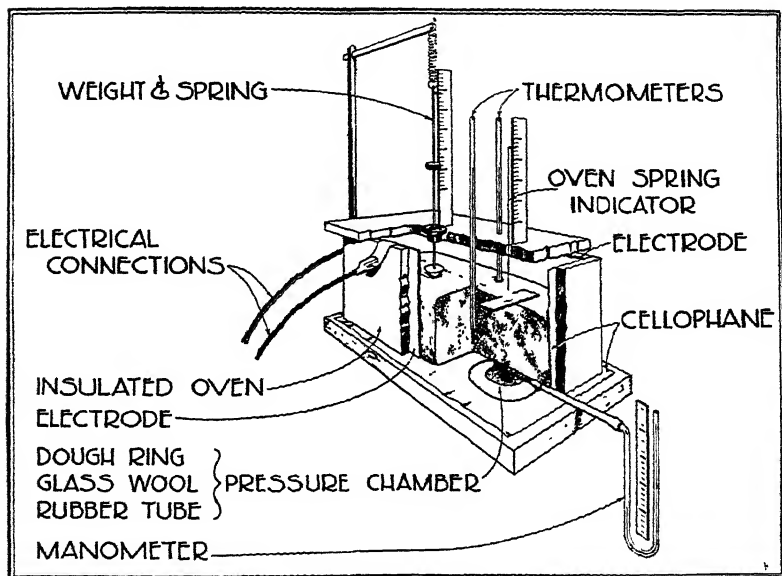


Fig. 1. An improved baking pan.

the changes in plastic properties of the dough. Lastly, a chamber was devised underneath the dough, communicating to a manometer which records changes in pressure within the dough structure itself, whereby the forces operating within the dough causing its expansion are indicated.

Numerous difficulties developed in the apparatus previously described. By changing the size of the pan to 9 inches long,  $3\frac{3}{4}$  inches wide, and 6 inches high, and reducing the current to 150 watts, more uniform heating was obtained. Electrode surface phenomena appeared to be causing unequal resistance and unequal distribution of heat and interfered with the conductivity-voltage relationship. This

difficulty was corrected by coating the electrodes with an alcoholic solution of quinhydrone which, acting as a chemical reservoir, would store up the reactions of the alternating current so that no local formation of gases occurred and perfect adherence of the dough mass to the electrode was obtained. This coating has given very satisfactory performance, as indicated by the reproducibility of results.

Numerous metal parts used in the earlier apparatus tended to short-circuit the current and have been entirely eliminated by making the entire chamber, except the electrodes and the falling weight, of non-metallic substances which are good insulators. For this reason the use of metallic thermometers was abandoned and glass mercury thermometers adopted, with increased accuracy as to reproducibility and with less lag in following the actual temperature of the dough. The falling weight was redesigned so that the portion penetrating the dough would present the minimum surface to the dough. It still has sufficient cross-section to make a tunnel through the dough which does not close above the weight and adhere to the shaft. Additional weight is carried on the shaft above the plunger to force it through all types of dough. This necessitates the supporting of the plunger by means of a spring, which partially supports the weight at the moment of penetration and totally supports it when the weight reaches the exact bottom of the pan. The weight of the plunger used when entering the dough is 46 g. Its diameter is 15 mm., with a tapered point at an angle of 60°. This spring also assures a vertical movement of the plunger.

The motion of the plunger is complicated not only by the effect of the spring upon the weight but by the upward motion of the dough during baking. It is necessary to correct the motion of the plunger in accordance with the motion of the dough and in proportion to the position of the plunger in the dough at each reading. The movement of the plunger through the dough and the upward movement of the dough (oven spring) are plotted in the same units so that their motion relative to each other can be seen.

To measure the pressure at the bottom of the chamber, difficulties were encountered in the earlier device because the bond between the dough and the bottom of the pan was often not sufficient to retain the pressure, and gases escaped. This difficulty has been obviated by the use of the heaviest non-waterproof cellulose cellophane obtainable. This cellophane has the property of adhering to dough with greater strength than the bonds within the dough substance itself. To further assure that the gases will not be lost from the chamber by passage underneath the dough, the chamber is surrounded by a ring of unleavened, unsalted dough. Such a ring of dough presents an additional

barrier to the passage of gas out of the chamber by any path other than through the test dough itself.

The skin which forms over the outside of a dough during proofing is a barrier which is penetrated by the plunger with difficulty. Because of the increased strength of the skin the pressure required inside of the loaf to cause expansion is increased. In order that the values measured be indicative of the true properties of expanding dough, the top of the loaf is slit just prior to inserting the instruments, thus eliminating this undesirable effect of the exterior skin.

In the work of Baker and Mize (1939) the following changes are reported to occur as the temperature of the dough is elevated:

(1) Increase in volume of the gases within the dough is caused by thermal expansion, by gas being driven out of solution and by the increased rate of formation of gas by the yeast. This expansion of gas in the dough produces pressure and thereby causes elastic and plastic extension of the dough, as evidenced by oven spring.

(2) A softening of the dough occurs during heating, as shown by the change in the rate at which a weight drops through the dough.

(3) This softening process is quickly arrested by the starch swelling at 136° F. The swelling of the starch produces the following changes in the properties of the dough because of the water removed from the other dough ingredients by the swelling starch: *First*, the starch granules by their increased volume become fixed in location and move about in the dough mass with difficulty as further stretching goes on. *Second*, water is taken from the gluten by the starch. The gluten properties are thereby strengthened, becoming more viscous and more elastic because of this dehydration. *Third*, the transfer of water in the system increases the electrical resistance and is accompanied always by a rise in voltage.

(4) The swelling of the starch is accompanied by a slight decrease in rate of temperature rise, indicating an increased absorption of energy. This reaction is largely completed within a very short period of temperature rise, though further swelling of the starch may occur during the continued heating of the dough.

(5) Destruction of yeast seems to begin during the same temperature range as starch swelling, but is not completed until the temperature is much higher.

(6) In desirable doughs, oven spring continues during and after primary starch swelling and is characterized by a rising pressure within the dough. The expansion of doughs, not previously arrested, ceases during gluten coagulation. At the usual rate of heating during baking, gluten coagulation does not perceptibly begin until after 165° F. has



been reached, and progresses slowly. Long-continued heating is necessary for complete coagulation of the gluten.

(7) Alcohol and water are distilled from dough, thereby furnishing an additional volume of gas which may produce pressure and which, by heat of evaporation, holds down the temperature during their distillation. These gases upon escaping from the bread sweep out carbon dioxide.

(8) There may be a marked increase in electrical resistance in many doughs toward the end of baking, particularly those which have been over-oxidized.

### Experimental

*Oven spring.*—Dough expansion during heating is determined by two properties in the dough: first, by the increase in volume of gas, and secondly, by the amount of gas which is retained.

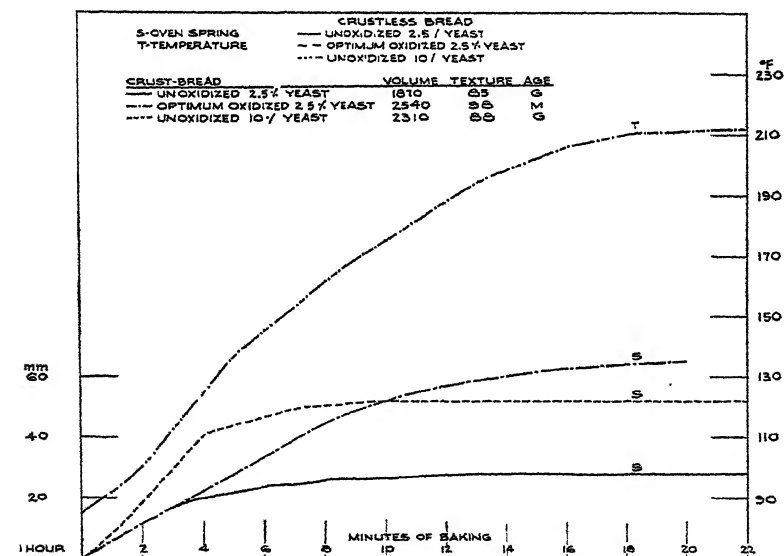


Fig. 2. Relation of oven spring to amounts of yeast and oxidation.

Figure 2 shows the oven spring of, first, an unoxidized, no-time dough; second, a similar dough modified by optimum amount of oxidation; and third, another similar dough modified by using four times as much yeast. The doughs containing different amounts of yeast increase in volume from the beginning of heating at markedly different rates. With the same amount of yeast, the unoxidized dough expands at the same rate as the oxidized dough until it reaches a point at which gas is not held in sufficient quantity to maintain that

rate of expansion. It is to be noted that the oxidized dough continues its oven spring during a long period of the heating cycle until the zone of gluten coagulation has been entered. To obtain this oven spring, it is necessary for the dough mass to continue its stretching and plastic flow after starch swelling has produced its profound changes upon the dough properties. It is such flow that enables a loaf to produce a smooth, shredded crust during oven spring.

The effect which any change in dough composition or handling will have upon oven spring depends upon whether the change alters gas production or gas retention. Those changes which affect gas production give oven spring differences which appear at once upon heating. Those changes which increase gas retention give oven-spring characteristics that appear during a later portion of the heating period.

The above doughs and all succeeding doughs, unless otherwise noted, are from the same Kansas patent flour baked as no-time doughs. The doughs were panned immediately after mixing and in all cases baked when proof height was reached. No-time doughs and patent flour were used for these experiments to show as far as possible the effects of each variable upon the dough without modification by fermentation and enzymatic action. This use of no-time doughs magnifies and makes distinctive their differences and simplifies the interpretation of results.

Before proceeding to the next chart it will be desirable to review briefly the work of Halton and Scott Blair (1937). Broadly these investigators and co-workers have found that dough quality is determined by the relation of viscosity to the modulus of elasticity. The viscosity should be as high as possible and should not fall too rapidly with addition of water or with fermentation or with increasing stress. They also state that a suitable modulus of elasticity is necessary. One can readily see that unless a dough is highly viscous the elastic properties do not come into play. If you increase the viscosity, the dough will stretch elastically. If the viscosity is too low, it will not stretch but will flow and run. A dough may have a fine elastic gluten but if its viscosity is low and the dough runny, it will not stretch. A green dough is of that type.

*Plunger-weight (W) and pressure (P) measurements.*—During the baking of the same three doughs shown in Figure 2 we measured the fall of a weight through the dough and the changes in pressure occurring while baking. In Figure 3 these measurements are superimposed upon those shown in the previous figure. It is to be noted that with the larger amount of yeast, substantially no difference occurred in the fall of the weight through the dough nor the pressure produced in the dough by the expanding gas. On the contrary, the same dough when

oxidized and baked with the smaller amount of yeast permitted the weight to drop more slowly and gave marked pressure characteristics. The pressure rose substantially throughout the entire course of the baking.

Reasoning from Scott Blair's ratio it is apparent that oxidation has increased the viscosity of this dough, whereas the increased yeast content has substantially no effect on this property in these no-time doughs.

It is particularly to be noted that the oxidized dough supported the falling weight so that there was substantially no motion through the dough until the softening produced by heat weakened its properties

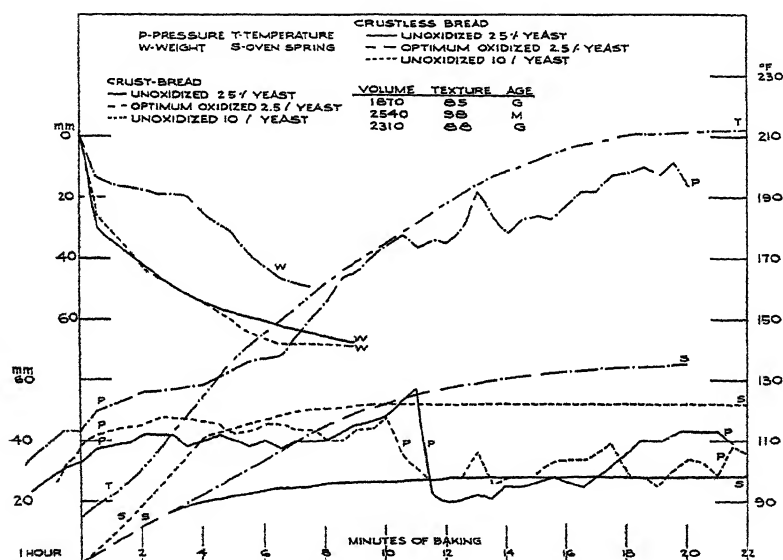


Fig. 3. Relation of oven spring, pressure, and weight to amounts of yeast and oxidation.

and the weight resumed its course downward. This period of arrested motion which because of oven spring is accompanied by an actual upward motion of the weight and thereby increasing force applied to the dough, suggests that the viscosity is so high in this dough that the weight is supported by the elastic properties of the dough and held there until the softening by heat permits the dough to flow and release the weight.

The movement of the weight shown here with the oxidized dough will usually be found characteristic of good doughs whether obtained by oxidation or by other means. The changes in dough which produce undesirable results are those associated with excessive softening and

loss of viscosity during heating. All doughs soften but those which produce desirable results are associated with a lesser amount of softening, indicating that they retain sufficient viscosity and elastic extension to carry the dough structure to the point where the swelling of starch by removal of water increases its strength.

Doughs which make good bread have a high initial pressure and exhibit a rising pressure while baking. If the pressure during the earlier stages of baking does not rise, poor bread is obtained. If the pressure does rise then good texture is retained in proportion to the duration of the rise and to the slope of the pressure curve. The steeper the slope and the longer its duration, the finer the texture found in the resulting bread. However, later graphs will show that on any type of pressure curve, should the pressure fall prior to or during starch swelling, then oven spring ceases and small volume is obtained. But good texture is retained if the pressure starts from a high level and the slope is an ascending one prior to the fall.

*Voltage measurements.*—Table I shows the influence of proof volume of a dough upon the voltage required to momentarily pass 150

TABLE I  
VOLTAGE REQUIRED TO FORCE 150 WATTS OF 60-CYCLE CURRENT THROUGH 540  
GRAMS OF DOUGH AT VARIOUS PROOF HEIGHTS

Time	Temperature	Proof height	Voltage
<i>Min</i>	<i>°F.</i>	<i>mm.</i>	
0	86	36	73
10	86	45	75
20	87	53	77½
30	87	62	80
40	88	73	82
50	88	82	85
60	89	92	87
70	89	100	88½
80	89	107	90½
90	89	112	91
100	89	112	92
110	89	112	91½
120	89	113	92
130	89	112	91½
140	90	108	89½

Note: Regular proof height is 90 mm.

watts of electricity through the dough. It is to be noted that the resistance of the dough progressively increases as the proof height increases. Inasmuch as the electrode surface utilized is constantly increased during proof, the increase in voltage can only be due to changes within the interior of the dough which increase the electrical

resistance. This is believed to be due to the increasing diameter of the expanding bubbles, which thereby causes the electrical path to become longer.

Voltage curves indicate the relative conductivity of doughs during the heating and show how the conductivity is affected by water, salt, dough composition, volume of the dough, and hydration of the dough ingredients at the starch swelling point and also toward the end of the heating period.

The suggestion in paper No. I of this series that a voltage rise in these curves was due to differences in gluten coagulation brought about by oxidation is not supported by further investigation. Following the method of Alsberg and Griffing (1927) no difference in alcohol solubility was found between oxidized and unoxidized cooked doughs, although these gave a wide divergence of voltage near the end of the heating period. Neither was there a difference found in degree of dispersion with sodium salicylate when used in a method suggested by Rich (1936) and modified for doughs by Dr. H. K. Parker, to whom we are indebted for these observations. This increase in resistance, however, must be associated with the transfer of water in the system and therefore suggests that oxidation has increased the hydration capacity of the dough.

The following charts show how dough properties are changed by varying the composition of the dough.

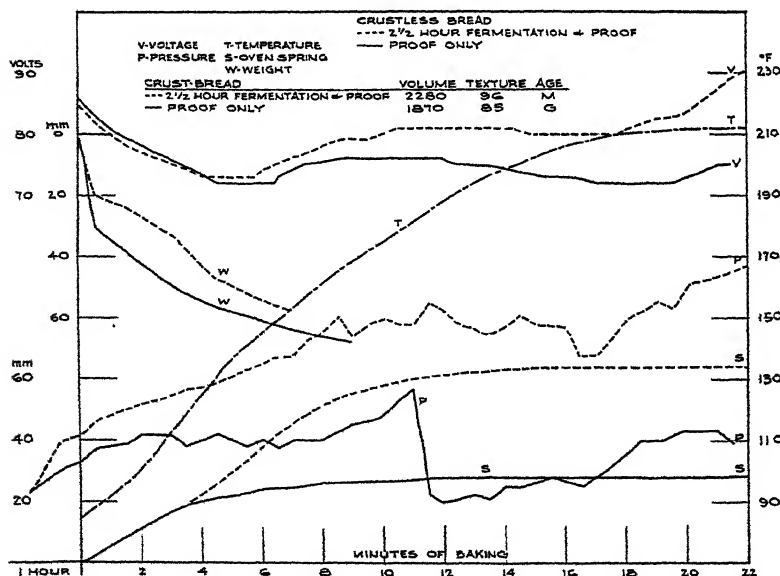


Fig. 4. Effects of fermentation on unoxidized dough.

*Effects of fermentation.*—Figure 4 shows the effects of fermenting a dough to which sufficient sugar was added. After two and a half hours of fermentation the dough was placed in the electric pan and handled as before. The fermentation has apparently changed the properties of the dough, as is shown by the graph. It has changed from a dough which had low viscosity and did not retain the pressure, to one that holds the pressure well. The rise in pressure is smooth until oven spring is almost completed, when it becomes irregular. The gas is now coming out of the dough in puffs. It seals up again, puffs out again, seals up again, and so on. The property of resealing after gas escape is a very important thing in dough.

The fall of the weight through the dough is changed materially by fermentation and shows a viscosity increase. The weight slows up quickly but heating again softens the dough and the weight movement is again increased and then slows as the starch swells. Apparently the effects of fermentation and oxidation are somewhat similar.

It is to be noted that the divergence in voltage which occurs is substantially paralleled by the divergence in volume of the two loaves except toward the end of the heating period.

*Effects of water.*—The ingredients in the doughs for Figure 5 were the same as the optimum oxidized dough in Figure 3, except for absorption. In Figure 3, 66% absorption was used; in the stiff dough in Figure 5, 61% water was added and the thin dough had 71% water therein. The stiff dough gave better texture and less volume. Both made good bread and gave a high initial pressure and steady pressure rise during baking. The steeper ascent of the pressure in the stiff dough indicates a more favorable viscosity-elasticity ratio and should give the better texture of the two. The movement of the weight also shows the stiff dough to be very viscous, so that the weight was supported for a long time at a high level and the softening by heat delayed. The thin dough had its viscosity and elasticity both lowered so much that the weight was not supported at any time. However its ratio is more favorable, as shown by the slower motion and distance traveled, than in the untreated doughs of lesser water shown previously in Figure 3.

The difference in conductivity is due chiefly to the difference in water content. The final voltage rise is probably due to the taking up of water by the dough ingredients. In the stiff dough this rise is higher because of its much lower content of free water.

*Effects of shortening compounds.*—Figure 6 shows three doughs, one baked with commercial hydrogenated shortening, one with refined cotton-seed oil, and the third with no shortening. These doughs were oxidized to make good bread and were identical in all respects except

for the change in shortening noted. The dough containing hydrogenated shortening gave a large, fine-textured loaf of commercial crust bread. The doughs with fluid shortening and no shortening gave small, very fine textured loaves of bread. The only outstanding differences among the three loaves were in volume. Similar volume and texture characteristics were obtained in the crustless test loaves as shown in the graph. All three loaves gave the same oven spring until divergence occurred, showing that the difference in volume is due to gas retention.

The pressure curves show that the loaf with the standard hydro-

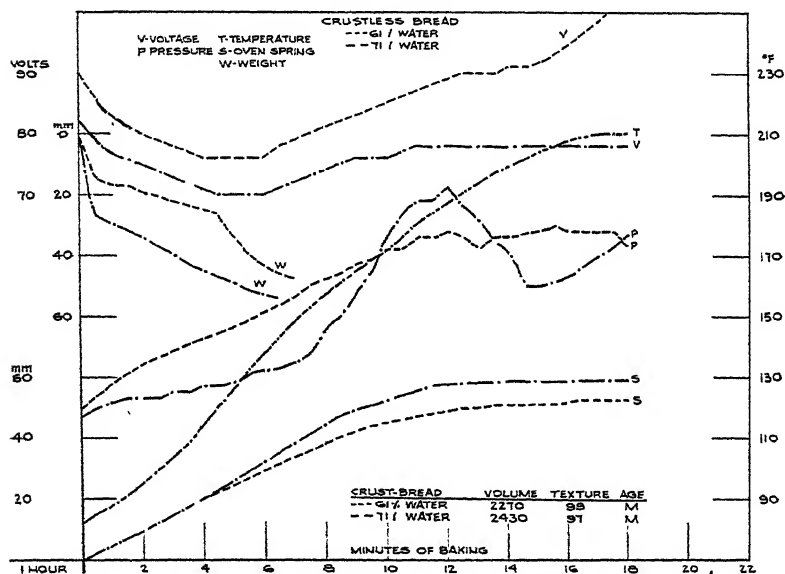


Fig. 5. Effects of water content on optimum oxidized dough.

genated shortening had the usual ascending pressure characteristic of good bread, whereas the two loaves which had the small volume followed also for a period the same ascending pressure, and then a marked drop in pressure occurred. At the same time the oven spring ceased. This drop in pressure continued downward until the swelling of the starch reinforced the properties of the dough and it resumed its ability to retain gas. Then the pressure built up rapidly and steadily to a very high level, with little increase in volume and continued through to the end of the baking period.

The problem here is, Why did these two loaves fail to hold pressure and gas during the critical softening period of the baking, whereas the loaf containing semi-solid shortening gave an entirely different per-

formance? The motion of the weight gives no satisfactory explanation. In all three doughs the falling weight performed in substantially the same manner, giving all the characteristics that would be expected of a dough of fine quality and showing that the elasticity-viscosity ratio of these doughs must be alike in all three cases.

This leads to the conclusion that the difference in these doughs is due to the character of the fats. Tests with other fats indicate that neutral liquid fats act like the cottonseed oil and that the action of semi-solid fats is similar to that of commercial hydrogenated shortening. One is led to speculate as to why this should be. The fine texture of all of these loaves shows that there is no coalescence, nor was the

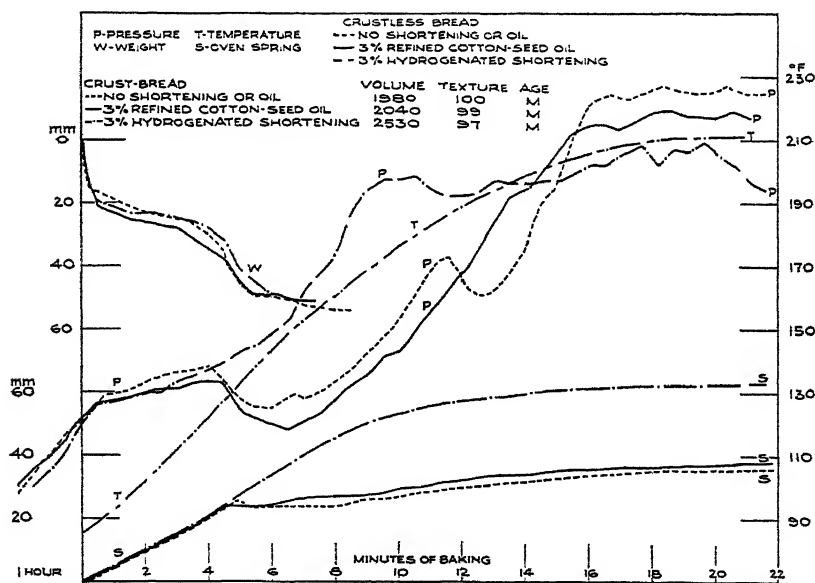


Fig. 6. Effects of shortening on optimum oxidized dough.

cell structure disrupted as was shown by their final ability to retain gas. Apparently gas must escape from these doughs during the softening period by diffusion through the cell walls; hence the difference in their ability to retain gas may be a difference of cell-wall porosity. Here is a clew: solid shortening may prevent cell-wall porosity, whereas fluid shortening or no shortening is unable to do so. Possibly the solid shortening is present throughout the proofing and early baking period as originally distributed in the mixture, whereas the fluid shortening disperses further during the proof. As the critical point in the dough is reached the semi-solid shortening melts and closes the pores so that gas escapes with more difficulty; hence the dough is



enabled to continue its expansion until starch swelling reinforces the entire structure. The liquid fat, being dispersed before baking, is unable to produce this effect.

### Summary

A method of testing dough described by Baker (1939) which gave means for indicating the electrical conductivity, the plasticity, the oven spring, the temperature, and the pressure required to extend the dough while being heated from 85° to 212° F. has been further investigated.

The apparatus has been improved, so as to prevent heat loss or gain, to produce more uniform heating of the dough, to assure a more constant conductivity-voltage relation, to measure more accurately the plasticity of the dough by means of a falling weight, and to indicate more accurately the pressure within the dough by the manometer attached to the redesigned gas chamber.

By the use of this redesigned apparatus it was found that changes which affect gas production in a dough give oven-spring differences which appear at once upon heating, while changes which increase gas retention give oven-spring characteristics that appear during a later portion of the heating period.

The voltage measured during baking is affected by the temperature, absorption, salt content, dough composition, volume of dough and hydration of the dough ingredients. However, where the compositions of doughs are similar except for fermentation or oxidation, differences in voltages are caused largely by changes in volume and hydration.

The motion of a weight through dough is controlled by its plastic properties and is greatly influenced by relationship of viscosity to elasticity. Oxidation, fermentation, and low absorption may decrease the movement of the weight through a dough and alter the properties sufficiently almost to arrest its motion. All doughs soften under the influence of heat, so that the motion, if arrested, is resumed and continues down until arrested by swelling of the starch.

The pressure measured, as here described, indicates the cell pressure in the dough. Desirable doughs develop high pressure in the proof; during baking the pressure rises continuously into the gluten coagulation period. The more rapid the rise in pressure, the better the bread obtained. Any interruption in the pressure rise is accompanied either by loss in volume or poor texture. The rapidity at which the pressure rises is controlled by the Scott Blair viscosity-modulus ratio. The steeper the slope, the higher is the viscosity.

During the baking of well oxidized, no-time doughs, made without shortening, a softening occurs as shown by the falling weight. This

is accompanied by a drop in pressure and the stopping of oven spring. Liquid shortenings used to the extent of 3% show no substantial alteration of these effects. On the contrary, doughs made with 3% semi-solid shortening, when the period of softening occurs, show no fall in pressure and no slowing of oven spring. This difference in behavior indicates that doughs containing no shortening or containing liquid fats may become porous, allowing the expanding gas to escape during the softening period, while doughs containing semi-solid shortening are able to retain much of the gas until after starch swelling and on into the zone of gluten coagulation.

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## THE PROTEINASE IN WHEAT FLOUR<sup>1</sup>

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Food Research Division, Bureau of Chemistry and Soils, U. S. D. A.

(Read at the Annual Meeting, May 1939)

Evidence of the occurrence of a proteinase in flour has been cited in a previous paper (Balls and Hale, 1936). Some of this evidence goes back to 1884 (Balland) and more has since accumulated. Contributing thereto were the extraction and purification of a proteinase from bran and whole wheat, and its recognition as an enzyme of the papain type, as reported from this laboratory (Balls and Hale, 1935 to 1938). The proteinase of sprouted wheat may prove to be the same or a very similar enzyme, for Mounfield (1938) in an investigation of its properties has observed that its action on edestin (though not on gluten) is accelerated by cyanide.

The conclusion that the proteinase in flour is also a papainase, like that obtained from whole wheat, follows logically from the behavior of paste, dough, and gluten toward oxidizing and reducing agents known to influence the activity of both papain and the wheat

<sup>1</sup> Food Research Division Contribution No. 432.

proteinase. Jørgensen has arrived independently and contemporaneously at this conclusion regarding the papain-like nature of the flour enzyme (1935, 1936, 1939). His work has definitely established the point at issue and the present paper aims only to furnish the type of direct evidence therefor that is based on the actual extraction of the proteinase from patent flour and the observation of its behavior in solution toward well-known activators and inhibitors of papain.

Like papain and the proteinase from bran, the enzyme isolated from patent flour is activated by sulphydryl compounds and inhibited by ascorbic acid, iodoacetic acid, cystine, and various oxidants of the bread-improver type. There seems to be no reason for changing the opinion expressed by Balls and Hale (1935, 1936, and 1938) that the action of air, bleaching agents, and bread improvers in modifying the baking properties of flour depends on a more or less complete inactivation of the proteinase.

### Extraction of the Enzyme from Flour

One kilo of unbleached patent flour was extracted for 24 hours at 0° with 4 liters of 10% sodium chloride solution containing a trace of cysteine. After being centrifuged in the cold the supernatant liquid, with still another trace of cysteine, was made 0.4 saturated with ammonium sulphate and left for 24 hours longer at 0°. The precipitate was then centrifuged out and discarded. The supernatant liquid was next made 0.8 saturated with ammonium sulphate and allowed to stand for an additional 24 hours at 0°. The precipitate was then filtered off and dried on a porous plate. The yield was 30 grams of dried material.

Ten grams of the dried preparation were dissolved in 60 cc. of a 15% glycerin solution containing a trace of cysteine, and dialyzed under pressure overnight against 15% glycerine solution at 0°. The inactive protein that precipitated during the dialysis was then centrifuged out. The resulting solution contained 1.06 mg. of protein nitrogen per cc. It represented, therefore, about a tenfold increase in purity over the original flour, but none over the original flour proteins. Such a preparation, however, possesses the great advantage that it can be used in a homogeneous system, thus excluding questions of extraction, diffusion, and adsorption on accompanying solids.

### Method of Estimation

The dialyzed solution served as a reference with which to compare the activities of other preparations of the enzyme. A set of empirical curves similar to those used for the estimation of the enzyme from

wheat bran was made, using various quantities of the dialyzed solution. Figure 1 shows the curves obtained, expressed in terms of the loss of viscosity per milligram of protein nitrogen in the enzyme preparation. Measurements were made after four different times of digestion.

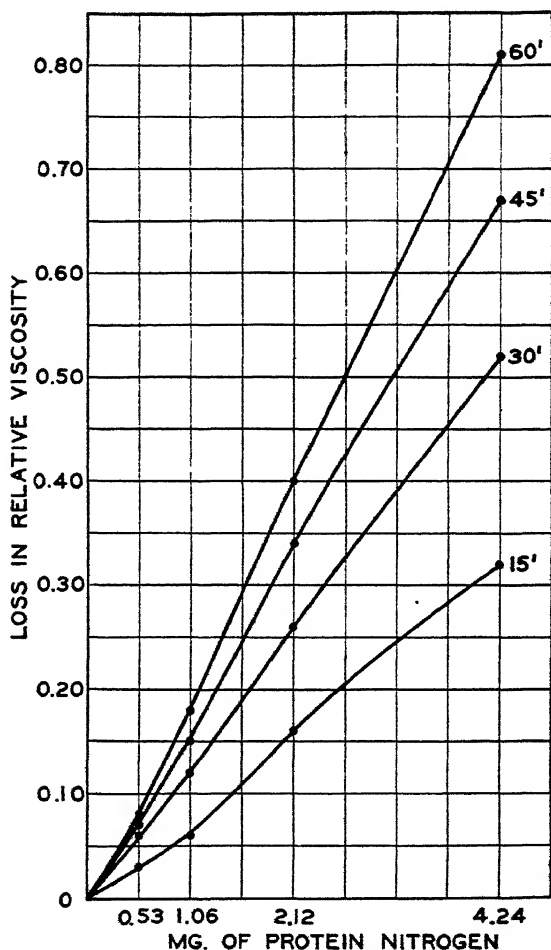


Fig. 1. Loss of viscosity per milligram of protein nitrogen in the enzyme preparation.

The apparatus, solutions, and method used for determining the change in the viscosity of gelatin are the same as those described in the previous paper (Balls and Hale, 1938), with the exception that the activity values are here expressed as milligrams of protein nitrogen in that quantity of the reference preparation showing the same activity.

### Properties of the Extracted Enzyme

The dialyzed preparation just described was used to determine the effect of several oxidizing and reducing agents on the activity of the enzyme. Activation by cysteine or cyanide and inactivation by iodoacetic acid and oxidizing agents have been observed to differentiate papain from the other types of proteolytic enzymes. It is not necessary to discuss the reasons for this behavior of papain when the question is merely one of identifying the enzyme. It is commonly held that the presence of a sulphhydryl group in the enzyme protein is essential to its activity.

The flour proteinase is activated by cysteine and inhibited by cystine, bromate, persulphate, metavanadate, and iodoacetic acid. Ascorbic acid was also found to be a powerful inhibiting agent, thus corroborating Jørgensen's results on whole flour (Jørgensen, 1935a). The data are given in Table I. No fundamental difference in behavior toward activating and inhibiting agents has been observed between the enzyme prepared from flour and that from bran or whole wheat. The isolated flour enzyme appears likewise to be a papainase.

TABLE I

#### ACTIVATION AND INHIBITION OF THE PROTEINASE FROM FLOUR

Activity values refer to mg. protein N in the amount of reference preparation showing the same activity.

Volume of enzyme solution cc.	Activity after treatment for 30 minutes					
	No additions	Cysteine	KBrO <sub>3</sub>		K <sub>2</sub> S <sub>2</sub> O <sub>8</sub>	
		10 mg.	1 mg.	2 mg.	1 mg.	2 mg.
1	0.8	1.1	—	—	—	—
2	2.0	2.3	0.5	0.2	0.3	0.3

Volume of enzyme solution, cc.	NaVO <sub>3</sub>		Iodo- acetic acid	Ascor- bic acid	Cystine	
	1 mg.	2 mg.	M/100 <sup>1</sup>	1 mg.	2 mg.	10 mg.
2	0.8	0.3	0.2	0.4	0.7	1.3

<sup>1</sup> Concentration in the enzyme solution.

A curious property of the enzyme from either source is that it is made irreversibly inactive by dilution with water, while the addition of cysteine before dilution seems to protect the enzyme, at least to some extent, from this decomposition. Table II summarizes the results from three experiments showing this behavior.

The proteolytic activity of the flour enzyme has also been observed with casein as shown in Table III. The method of determining casein digestion was that of titration in alcohol after incubation of the protein and enzyme at pH 5.0, as is frequently done in the assay of papain (Balls, Swenson, and Stuart, 1935). While considerable splitting of casein occurred in two hours, very much larger values were obtained in 20 hours. As with the preparations from bran, this is an indication, although no definite proof, that a peptidase may accompany the proteinase.

TABLE II

INHIBITION OF THE CRUDE FLOUR PROTEINASE BY DILUTION WITH WATER AND PROTECTION THROUGH THE PRESENCE OF CYSTEINE

Treatment	Activity— mg. protein N in corresponding amount of reference preparation
1 g. prep. B (from bran) dissolved in 39 cc. water; thereafter 100 mg. cysteine added. Assay on 4 cc. after standing 30 min.	0.0
1 g. prep. B mixed with 3 cc. water containing 100 mg. cysteine. After standing 30 min. diluted to 40 cc. with water. Assay at once on 4 cc. of the dilution.	1.1
1 g. prep. C (from bran) dissolved in 49 cc. water; then 100 mg. cysteine added. After standing for 30 min. 4 cc. was analyzed.	0.0
1 g. prep. C mixed with 2 cc. H <sub>2</sub> O containing 100 mg. cysteine and allowed to stand 30 min. It was then diluted to 50 cc. and 4 cc. analyzed at once.	1.6
500 mg. prep. 2 (from flour) dissolved in 10 cc. water; 1 cc. of this + 10 mg. cysteine stood for 30 min.	0.1
500 mg. prep. 2 dissolved in 5 cc. water; 0.5 cc. of this + 10 mg. cysteine stood for 30 min.	1.0

TABLE III

CASEIN DIGESTION AT 35° C. BY CYSTEINE-ACTIVATED FLOUR PROTEINASE

Amt. enzyme	Substrate	N/20 KOH after	
		2 hrs.	20 hrs.
cc.		cc.	cc.
None	Casein alone	0.00	0.00
2	No casein	0.00	0.00
2	6% casein + 10 mg. cysteine	0.85	3.00
5	6% casein + 10 mg. cysteine	1.25	5.05

### The Amount of Proteinase in Flour

If a small quantity of flour made into a thin paste with a cysteine solution is added to gelatin, a significant loss in viscosity takes place. The results of such an experiment are shown in Table IV. They probably indicate little more than a lower limit to the amount of

proteinase present. While the flour proteins were evidently dispersed by the cysteine, they probably reprecipitated when mixed with the gelatin and buffer. This would explain why increasing the fineness of the flour by pulverizing it in a ball mill had no marked effect on the results. When gluten alone instead of whole flour was used, the reprecipitation of the dispersed protein was easily noticed. It usually clogged up the viscosimeter. Furthermore the flour proteins probably compete so successfully for the enzyme with the gelatin that the digestion of the latter is greatly hindered. Thus, when a small amount of crystalline papain was also added to the flour the rate of gelatin liquefaction was not increased, although the quantity of papain was sufficient to digest the gelatin in the absence of the flour. This does not necessarily imply that the papain was inactive, but rather that its activity was here confined almost exclusively to the flour proteins.

TABLE IV  
APPROXIMATE AMOUNT OF PROTEINASE IN UNBLEACHED PATENT FLOUR

Sample	Activity— mg. protein N of reference preparation	
	<i>After min.</i>	
50 mg. flour, untreated, mixed directly in the viscosimeter with gelatin containing 10 mg. cysteine. Total vol. 10 cc.	15	1.0
	30	0.8
	45	0.8
	60	0.8
50 mg. flour treated with 10 mg. of neutralized cysteine HCl in a volume of 1 cc. for 30 min. The mixture then added to the gelatin in the viscosimeter as usual.	15	1.1
	30	0.9
	45	1.0
	60	0.9
50 mg. flour, pulverized in ball mill, treated with 10 mg. neutralized cysteine HCl in a volume of 1 cc. for 30 min., then assayed as usual.	15	0.8
	30	0.7
	45	0.7
50 mg. flour, plus 0.001 mg. active crystalline papain, treated with 10 mg. neutralized cysteine HCl for 30 min., then placed in viscosimeter.	15	1.0
	30	0.7
0.001 mg. active crystalline papain + 10 mg. neutralized cysteine HCl tested directly after mixing.	15	1.5
	30	1.2

The data in Table IV indicate, therefore, only the least amount of proteinase that may be present. Nevertheless the flour used reduced the viscosity of gelatin at about the same rate as one fifty-thousandth of its weight of crystalline papain. This quantity of enzyme seems in fact surprisingly large, when one considers the effect of a trace of papain added to dough. One part of commercial papain to twenty

thousand parts of flour may completely liquefy a dough, and a quarter of this quantity of the crystalline enzyme should also suffice. If, as seems reasonable, this marked change is caused by scarcely doubling the proteinase, it follows that the amount naturally present is of no slight importance. There is without doubt enough to produce disastrous effects if by mischance the enzyme should be activated—a situation that can conceivably arise in several ways, for example through the autolysis of dead yeast cells and the liberation of their glutathione. The disintegration of gluten by incubation with cysteine has been previously described (Balls and Hale, 1936a). It was not possible to demonstrate that any proteolytic action had taken place during this treatment; yet proteolysis was by no means ruled out. The gluten undergoes a remarkable decrease in viscosity by the addition of cysteine and although it can be re-coagulated with salts, the resulting gluten is thereafter of very poor quality. The evidence now seems to point to the conclusion that the proteinase is involved in this dispersion of gluten, although the action definitely stops short of an extensive hydrolysis. The data corroborate precisely Jørgensen's statement (1936) that "flour contains a powerful but latent proteinase."

The amount of proteinase in flour is sufficiently striking to justify considerable speculation as to its effect. It is obvious that no thorough-going breakdown of the protein occurs in normal dough; therefore it is reasonable to look for the effects of the flour proteinase in the direction of protein modification, rather than extended hydrolysis. To a greater or less degree all proteinases appear to have the property of producing a clot with various proteins. The clotting of milk by chymotrypsin and of blood by papain are well-known examples. The formation of gluten also has the appearance of being an enzymic clotting. The experimental proof of such a hypothesis would be quite a difficult matter, particularly since it cannot be claimed that proteolysis of flour protein is the only factor involved. Contact between the protein particles is also necessary and some pressure must be applied to cause them to coalesce. Hence the gluten mass is not formed in thin pastes but may be prepared therefrom by centrifuging. However, such behavior in a heterogeneous system like dough does not preclude the possibility of a chemical change in the protein.

### Summary

A proteinase was extracted from patent flour, but not successfully purified thereafter. Examination of its behavior toward oxidizing and reducing agents has led to the conclusion that it is an enzyme of



the papain type. There is no reason at present to believe that it is different from the enzyme obtained previously from bran and whole wheat. The flour proteinase was found to be activated by cysteine and inactivated by iodoacetic acid and a variety of bread improvers.

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(Aug. 28, 1939)

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## BOOK REVIEWS

**Modern Cereal Chemistry.** By D. W. Kent-Jones. Third edition. The Northern Publishing Company, Ltd., Liverpool, England. 724 pages. Price in United States \$7.15, at Broomhall's Agency, 230 Produce Exchange, New York.

To all who are interested in keeping thoroughly informed and up-to-date in the rapidly expanding field of cereal technology, the appearance of Dr. Kent-Jones' third edition of *Modern Cereal Chemistry* is an event of major importance. From a modest beginning in 1924, the first edition of 324 pages has now been expanded into a comprehensive, authoritative, systematic, and up-to-date treatise of 724 pages.

Most of the material has been revised, expanded, and rewritten, and four new chapters have been added. Especially timely is the chapter on "Dough Testing Machines," dealing with modern physical methods used in flour and dough testing. Another noteworthy addition is the chapter on "The Microbiology of Cereals," contributed by Dr. A. J. Amos. Two other important new features are chapters, respectively, on "Flour for Purposes Other than Bread Making" and "Cereal and Balanced Rations for Live-stock."

In a large chapter dealing with "Methods of Analysis" the author has fully and adequately described and discussed the details, purposes, and merits of the principal chemical methods, with their modifications for special requirements, that are now available for use in the cereal laboratory. The baking test, with related matters, is appropriately dealt with in a separate chapter.

The author's thorough familiarity with past as well as with current literature in the field of cereal technology is amply proved by his references and bibliography containing approximately 600 citations, which have been selected with care, and which include papers published in 1938. Some will take issue with the selection of certain papers and with the omission of others, but all will agree that in the main an excellent sense of discrimination has been shown.

The book is written in simple, clear, and understandable English, and in a style which reflects the ability, energy, and enthusiasm of the author. Although some may be inclined to criticize the emphasis on certain processes and to question some interpretations placed on various features of flour and dough behavior, it is the opinion of the reviewer that controversial issues are for the most part handled with sound judgment and with a degree of impartiality that is highly commendable. No cereal technologist, whether a beginner or an "old-timer," whether in research or control work, can afford to be without a copy of *Modern Cereal Chemistry*, which the reviewer considers to be the most complete, up-to-date, and authoritative work on wheat and flour technology now in existence.

M. J. BLISH

**Das Roggenmehl.** By Arne Schulerud, Oslo. Published by Verlag von Moritz Schaefer, Leipzig. 149 pages. Price 9.5 marks.

This book on rye flour will be welcome to anybody who is interested in this cereal. In a concise form it gives the present status of our knowledge of rye flour and its baking characteristics. The subject matter is divided into the following six chapters:

I. Rye varieties and rye flour milling	7 pages
II. A review of the chemistry of rye flour	28 "
III. Development and physical structure of the dough	38 "
IV. The influence of chemical and physical factors on the characteristics of rye flour	27 "
V. Bacterial action and yeast fermentation	15 "
VI. What happens during the rye flour baking process	32 "

Chapter I. Supplies statistical data regarding rye culture over the world and a discussion of the rye types and their characteristics in different countries. There is very little mentioned about the rye milling process.

Chapter II. Deals with the importance of the following chemical factors: moisture, ash, protein, fat, and carbohydrates in rye flour. The carbohydrates are especially emphasized on account of the important role they play in the rye baking process. Staling is shortly discussed and attention is called to the importance of acidity. A discussion of the principal enzymes in rye flour closes this chapter.

Chapter III. Contains a discussion of the physical properties of a rye dough, such as consistency, elasticity, plasticity, viscosity, etc. The application of the farinograph to rye doughs is discussed. There is also an interesting treatment of the viscometric phenomena of the rye flour water system, when subjected to gradually increasing temperatures. The possibilities of the Brabender amylograph are discussed in this connection.

Chapter IV. Deals with the influence of salts and some organic acids on rye doughs, also the effect of heat and enzymatic action on the baking quality of rye flour.

Chapter V. Contains a general outline of the happenings in sour dough and yeast-fermented doughs and a discussion of the connection between gas production, yeast quantity, and available sugar.

Chapter VI. Treats of the more technical phases of the rye bread baking process, such as mixing, kneading, dividing, and molding. This chapter contains an interesting description of the processes occurring in the fermenting dough and what happens when the dough is subjected to the heat of the oven until it is ready to be taken out in the form of bread.

A list of 61 references from the literature closes the book. Its author is an outstanding authority on the subject of rye and rye baking and the prospective reader may be assured that he will be in reliable company. The publisher comes in for praise as regards the general appearance of the book.

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## PSYCHO-RHEOLOGY IN THE BREAD-MAKING INDUSTRY

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(Read at the Annual Meeting, May 1939)

The importance of the psychological aspects in the problem of the judging of flour strength has already been pointed out by Katz (1937). In many parts of the world, the quality of a flour is still assessed by a baking test, the conditions of which are defined by the subjective judgments of the baker, and even where the control is entirely mechanical, the final criterion of quality is the satisfaction or dissatisfaction which the baker expresses, in relation to his own standards and interpretation of public taste. This taste varies widely in different countries, and even in different parts of the same country, and there is also a large personal factor differentiating even the best of bakers within quite a small geographical area.

Katz has pointed out that the physical factors judged by feel are not necessarily the same as those chosen by the physicist in order to classify his data. He points out that "the psychological properties, *e.g.* body and spring, do not correspond to the physical properties of viscosity, elasticity, etc., but are rather the result of a complicated cooperation of the different senses of the skin, the muscles, the sinews, and the joints." It is of interest in many industries, and perhaps most of all in the bread-making and dairy industries, to study the nature of the physical properties which can be assessed by touch, with what order of accuracy these can be determined, and in what manner the experienced technician is at an advantage by comparison with the ordinary citizen. It is with these three problems that the present paper is intended to deal.

Katz quotes a letter from the present writer, as follows: "It seems as though differences in certain physical properties are much more easily observed by feel than is the case with others. For example, we suspect that the hand is comparatively sensitive to changes in elasticity modulus, but insensitive to changes in viscosity."

It therefore seemed advisable to find out what percentage differences could be detected in the viscosity of a series of true fluids, and in the compression moduli of elastic solids. Scott Blair and Coppen (1939) have described experiments on about a dozen subjects having widely differing education and technological experience, and it appears that the smallest differences in viscosity which can be distinguished significantly more often than can be accounted for by chance, vary only a little as between different subjects. Bakers were not tested, but experienced cheese graders showed no more sensitivity than science graduates, graduates in arts, or subjects who had had only very simple education.

It is difficult to decide on the best single criterion from a batch of data such as these, but, merely for convenience, we will define the threshold for viscosity ( $\Theta\eta$ ) as the percentage change in viscosity which can be correctly given 80 times out of a hundred. The mean value of  $\Theta\eta$  under the conditions of our experiments, using Californian bitumen diluted with oil, and having a viscosity of the order of  $10^6$  poises (*i.e.* a hundred million times that of water) is 30%. The viscosities of the bitumen samples were determined by the method of rheograms first described by Schofield and Scott Blair (1932, 1933, 1933a, 1937) for use for flour doughs.

A similar experiment was carried out with rubber cylinders containing different amounts of "filler" which behaved as approximately elastic solids differing slightly in compression moduli, the compression moduli being of the order of  $1.5 \times 10^7$  dynes/cm<sup>2</sup>. The psycho-physical technique is being described elsewhere but the results will be of some interest to cereal chemists.

Unlike viscosity, the "threshold" for compression modulus ( $\Theta\kappa$ ) is by no means the same for different subjects, though again the skilled craftsman is at no advantage. Some subjects have decidedly lower  $\Theta\kappa$  values than others. Although our best two subjects were both routine analysts, the work is still at too early a stage for it to be possible to draw definite positive conclusions as to the relationship between education or training, on the one hand, and sensitivity to changes in elasticity moduli, but the negative conclusion that the experienced craftsman is not above the average is definite. It is also found that the threshold for compression modulus is only about one-third of that for viscosity. This means that, as suggested many years ago in my letter to Katz, elastic properties are more accurately differentiated than viscous properties. Perhaps this is true because it is easier to judge firmness statically in elastic systems. If a given stress is maintained, the deformation remains constant as long as the subject likes, whereas for a viscous material, an extra dimension is introduced,

and judgments must be made dynamically. If stress is kept constant, the sample keeps on deforming at a constant rate. The question is, however, still not fully understood.

Flour dough, and for that matter cheese, for which these experiments were primarily designed, are not true fluids like some of the bitumens, nor elastic solids like rubber (even though the consumer may occasionally doubt the last statement in the case of cheese!). They are intermediate in properties between the two, and Schofield and Scott Blair (1932, 1933, 1933a, 1937) have proposed equations containing an elastic and a viscous term (Hookian and Newtonian terms) to account for the experimental facts. Other workers, notably Bohn and Bailey (1936, 1936a), have also worked on similar lines.

Purely from the rheological point of view, this type of treatment is both practically useful and theoretically sound, but, as Katz has pointed out, the physical composites judged by technicians when handling a material like dough are by no means necessarily the same as those usual to the physicist. The conception of viscosity was evolved to explain the behavior of a material like water, which flows just twice as fast when pushed twice as hard; and that of elasticity was introduced by Hooke in his famous law, *ut tensio ut vis*, which is so nearly true for the recoverable deformations of metals and the like. When a baker judges "firmness" or "spring," he does not, in fact, divide his sensations into viscous and elastic parts, but forms a judgment based on the general behavior of the dough when handled. These facts suggest the need for a new line of approach in the rheological treatment of data for materials like dough. The author is in process of developing such a treatment, based on the following general considerations.

If a subject is asked to say which is the firmer of two viscous bitumens, he interprets "firmness" in terms of viscosity, whereas if he is comparing rubbers, he judges entirely by compression modulus. When given a bitumen to compare with a rubber, he is, in effect, trying to compare properties which have different dimensions. It is as if he were asked "Which is the greater, an hour or an elephant?" Most subjects do not realize this but make some kind of mental synthesis, and if the two stimuli differ greatly in terms of their own absolute standards, they show no hesitation in giving an opinion. Thus, to take the extreme case quoted, most non-scientific people at least would say that an elephant is bigger than a second, but might hesitate when asked to consider an hour or a day. In the case of rubber having a compression modulus of about  $1.5 \times 10^7$  cg. units and a bitumen of viscosity about  $5 \times 10^6$  cg. units, the subject will generally give the bitumen as the firmer if he compresses for only half a second, but the rubber, if allowed to compress for four seconds.



Subjects (including, of course, bakers) judge firmness by some entity of inconstant dimensions, the dimensions depending on "spring," and this suggests a line of approach on the rheological side. In a viscous material, the shearing stress is proportional to the strain multiplied by the time of application taken to the power of minus one. In an elastic solid, the time does not enter, or we may say that its exponent is zero. In both cases the constant of proportionality expresses firmness in the subjective sphere. May we not assume a similar state of affairs in a material like dough?

In such a case the dimensions of "firmness" will depend on the exponent of time. When this is zero, we have an elastic solid, and firmness becomes its modulus, whereas at the other extreme, the exponent being  $-1$ , the material is a true fluid, and its firmness is judged by viscosity. The exponent is, of course, a measure of "spring." The more "lively" the dough, the nearer the exponent approaches zero; the more "dead" the dough, the more closely it approximates to  $-1$ .

The connexion with the actual facts of subjective judging is so good that one does not feel disposed to allow the dimensional difficulties to stand in the way of the formulation of an equation along these lines, especially since a logarithmic form of equation will eliminate the difficulty at least formally. Such an equation will be developed elsewhere.

The last problem to be discussed is the nature of the advantage (if any) which the skilled craftsman has over the ordinary citizen in judging the rheological properties of materials.

From the experiments already described, it seems unlikely that there is any advantage, either inborn or developed, in the direct capacity to judge small rheological differences and one is left with two further possibilities.

It would be only natural to expect that, even if the brains of two subjects received the same impulses as the results of a test, the brain which was already armed with a wide knowledge and experience of the bread-making industry would be able to use the information obtained more effectively than would that of a person altogether ignorant of the subject. But there is also another way in which experience may help. In comparing the firmness of two materials, for example, we have seen that the length of time may drastically influence the judgment. If the materials are doughs, which show all the complex phenomena of work-hardening, structural viscosity, and elastic hysteresis, the exact conditions of stress and deformation will also be very important. If the comparison is not made directly, but after a considerable interval of time, two factors become important: (1) the

capacity to recognize rheological criteria previously met with and (2) the capacity to reproduce the movements of the hand used to make the test. It may well be that one type of movement is better than another. Is the expert using the best method, or is he using the same method each time, but not the best? By studying his movements we may hope to help him in this respect.

Head (1920) suggests that the sequence of any series of movements is controlled by mental pictures<sup>1</sup> of the postures involved in the movement, so that each posture must be considered in relation to the movement as a whole, thus introducing a kind of hysteresis. The existence of these mental pictures, which are called "schemata," enable us to perform without conscious thinking all the complex movements of everyday life. These ideas have been much developed by Bartlett (1932) and he has further suggested that they may be in some manner applicable to the problem of the psychological basis of craftsmanship such as we have been discussing.

#### Acknowledgments

I am indebted to many friends for help both in the experiments and theories discussed in this paper, and especially to Miss Coppen, who is carrying out these experiments with me, and with whom I have discussed these questions on many occasions.

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<sup>1</sup> One is not conscious of the presence of these mental pictures.

## A STANDARDIZED WOHLGEMUTH PROCEDURE FOR ALPHA-AMYLASE ACTIVITY<sup>1</sup>

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Since its adoption as a quantitative procedure by Wohlgemuth (1908) the amylolytic destruction of the ability of starch to give its characteristic blue color with iodine has been extensively used in measuring a certain type of diastatic activity. The hydrolytic action measured by this method has been characterized as dextrinization. The amylase component of malt which can readily bring about this change has been designated the dextrinogenic amylase, or more recently alpha-amylase. Based on the hypothesis that beta-amylase, the saccharogenic amylase of malt, plays little if any role in starch dextrinization, the rate of dextrinization as measured by the Wohlgemuth method has been generally accepted as exclusively a measure of alpha-amylase activity.

Ohlsson (1926, 1930) recognized that beta-amylase activity must be considered in the early color changes of the Wohlgemuth method but believed its action insignificant in the determination of his "x-values." By "x-value" he refers to dextrinization as measured at a point just preceding the final "colorless" end point with iodine. Holmberg (1933) recognized that some of the color change is influenced by the presence of beta-amylase. The roughly additive function of a mixture of alpha- and beta-amylases in starch saccharification was pointed out by Ohlsson (1926) and again by Freeman and Hopkins (1936). Blom, Bak, and Braae (1937) demonstrated the overlapping effects of alpha- and beta-amylases in starch dextrinization as well as saccharification. Finally Hanes and Cattle (1938) in an investigation of starch hydrolysis by mixtures of alpha- and beta-amylases found that "the rate of destruction of the iodine coloring property is by no means an exclusive function of the amount of the alpha-component."

If indeed beta-amylase strongly affects rate of dextrinization, and if the degree of effect varies with the quantity of beta-amylase present, it is obviously essential to eliminate or control this variable in any procedure intended for the exclusive and trustworthy measurement of alpha-amylase. An examination of possibilities in this direction has been undertaken, with results and conclusions as herewith reported.

<sup>1</sup> Published with the approval of the Director as paper No. 235, Journal Series, Nebraska Agricultural Experiment Station.



convenience it is desirable to avoid methods based on the selective destruction of alpha-amylase. The obvious preference involves the use of an extract of a sound ungerminated cereal grain endosperm. We have found non-diastringed hard winter wheat flour to be an excellent source of beta-amylase. It is recommended that an unsupplemented hard-wheat flour of high beta-amylase activity and known freedom from alpha-amylase be used as the source material.

To prepare the beta-amylase solution extract 400 g. of hard-wheat flour with 1,000 cc. of water for several hours at room temperature. Centrifuge and filter through cotton. When saturated with toluol and stored in a refrigerator this solution retains adequate activity for months.

The proposed addition, 24 hours before use, of 1 cc. of beta-amylase solution for each 100 cc. of buffered starch solution is more than adequate to cover any normal range of variation in beta-amylase activity of soft-wheat flours. However a possibility which must be guarded against is the presence of significant alpha-amylase activity in the hard-wheat flour extract itself. Most non-malted hard-wheat flours appear to have insignificant alpha-amylase activity but occasionally one is encountered which contains an appreciable amount of the alpha component. To determine whether or not the preparation is free from alpha-amylase perhaps the simplest procedure is to compare the dextrinization times for duplicate samples of malt extract, one acting on buffered starch to which an excess of beta-amylase (5 cc. of a 2 : 5 extract) is added simultaneously with the malt extract, the other on buffered starch which has received the same amount of beta-amylase two hours previously to the determination. The presence of alpha-amylase would result in the starch treated for two hours requiring less time for dextrinization than that receiving simultaneous addition. If there is significant difference the sample of hard-wheat flour must be discarded and another one tried.

### Procedure

*Preparation of the standard.*—Pipette 5 cc. of iodine solution (A) into a 15-cc. comparison tube.<sup>2</sup> Add one cc. of dextrin solution and shake. This standard should be prepared immediately before a series of determinations is to be made and serves without need of replacement for half a day.

*Determination of alpha-amylase.*—To 20 cc. of the buffered alpha-amylodextrin in a 50-cc. Erlenmeyer flask add 5 cc. of water and place in the 30° C. water bath. After a few minutes add 5 cc. of alpha-amylase solution to be tested. At appropriate time intervals add 1 cc.

<sup>2</sup> 15-cc. graduated centrifuge tubes serve as convenient comparison tubes.

of the hydrolyzing mixture to 5 cc. of dilute iodine solution (B) in a comparison tube, shake and compare with the standard. Color comparisons are made before a lightly screened 100-watt "daylight" bulb.

During the initial stage of the reaction it is convenient to pour approximately 1 cc. of the reacting mixture into the comparison tube. As the end point is approached the addition must be made accurately by means of a pipette. It is convenient to keep a series of tubes each containing an exact volume of 5 cc. of dilute iodine solution in readiness for testing.

For accuracy and convenience it is essential that the minimum time for dextrinization be not less than 10 minutes. With malt extracts these requirements are usually satisfied by using a 5-cc. aliquot equivalent to 0.05 g. of malt. If desirable this volume may be varied. The use of a larger or smaller volume of extract necessitates appropriate changes in the volume of water added so that the final volume of the reacting mixture is always 30 cc.

From the time interval necessary for dextrinization and the weight of malt represented by the extract aliquot taken, alpha-amylase units may be easily calculated. For example if an aliquot equivalent to 0.05 g. of malt dextrinizes 20 cc. (0.4 g.) of starch in 15 minutes the alpha-amylase activity is represented by  $\frac{0.4 \times 60}{0.05 \times 15}$  or 32 alpha-amylase units.

#### Application of the Standardized Procedure

The determined alpha-amylase activity of a malt will depend to some extent on the method of obtaining the extract. The conditions which we have found most suitable and convenient are as follows. Grind the dry malt sample finely in a burr mill. To one g. of the ground malt add 100 cc. of water and extract at 30° C. for one hour. At the end of the hour transfer to a centrifuge tube and centrifuge for 5 minutes. Filter the centrifugate rapidly through cotton. The extract is now ready for use.

A few trials were carried out to determine the influence of fineness of grinding and the time and temperature of extraction on the alpha-amylase activity of the extract. Within normal limits variation in fineness of grinding proved insignificant. Standardizing the time of extraction proved somewhat more important. In one instance a 60-minute extraction showed 40.0 alpha-amylase units as compared to 38.4 units for a 30-minute and 36.9 units for a 15-minute extraction. Extraction times of longer than one hour at 30° C. give slight increases in alpha-amylase activity but not large enough to justify the extra time involved. The significance of extraction temperature is well

illustrated by the data for one malt which by extraction for an hour at 30° C. showed 50.5 units of alpha-amylase activity. A duplicate sample, with the exception that it was extracted at 20° C., gave a value of 40.8 units.

A necessary precaution is that of determining the activities of the malt extracts as soon after extraction as possible. No significant loss of activity was observed over an 8-hour period but at the end of 48 hours, even when stored with toluol in a refrigerator, about 8% of the alpha-amylase activity was lost.

When the above procedure is observed duplicate malt samples may be ground, extracted, and run independently with less than 5% deviation between their measured alpha-amylase activities.

Data for the extracts from eight whole dry malts are shown in Table II. Several of these samples were supplied by the late Dr. D. A. Coleman, the remainder by the A.A.C.C. Committee on Malt Analysis. Dextrinizing and saccharifying activities of the malt extracts were determined as well as the activity attributable specifically to alpha-amylase.

TABLE II  
STARCH-DEGRADING PROPERTIES OF VARIOUS MALTS

Malt	Approx. Lintner value	Saccharification		Dextrinization		Alpha-amylase		
		Starch conversion		Time to end point	Relative value	Time to end point	Relative value	Alpha- amylase units
		15 min.	60 min.					
	<i>Degree</i>	<i>%</i>	<i>%</i>	<i>Min.</i>		<i>Min.</i>		
A	159	56.0	74.9	22.0	100	12.3	100	39.0
B	139	51.0	73.6	23.5	94	12.1	102	39.7
C	108	38.0	73.3	32.3	68	16.5	75	29.1
D	94	37.4	73.3	30.0	73	12.5	98	38.4
E	72	30.3	71.8	33.8	65	12.8	96	37.5
F	51	21.3	65.1	70.0	31	27.5	45	17.5
G	50	18.4	59.3	54.0	41	18.0	68	26.7
H	23	13.0	41.9	81.5	27	18.5	66	26.0

All determinations were run in a comparable manner using 20 cc. of 2% buffered soluble starch, the equivalent of 0.05 g. of malt, a total volume of 30 cc., and a 30° C. temperature as the conditions for reaction. Dextrinization and saccharification as such were of course determined without the addition of any increment of beta-amylase.

The Lintner values of Table II are in some instances the averages of those reported by a number of collaborators, in others the values supplied with the sample. Saccharification is expressed as percent of starch converted to maltose in the time interval stated. Dextrinization

is reported as the time interval necessary for the unsupplemented malt extract to reach the red-brown end point. Alpha-amylase activity is reported both in terms of the time necessary for the supplemented extract to reach the end point and as alpha-amylase units. For both dextrinization and alpha-amylase activity relative values are given with the results for sample A as 100.

The data shown in Table II confirm those of Blish, Sandstedt, and Kneen (1938) in as much as there is an excellent correlation between percent starch converted in 15 minutes and Lintner value. There is likewise a decided tendency for the dextrinizing activity to correlate closely with saccharification.

Turning now to specific alpha-amylase activity it is obvious that it does not correlate well with either saccharification or dextrinization. The general tendency is for those malts in the upper range of Lintner values to have a high alpha-amylase activity. However, malts A and D while representing a wide spread in saccharifying and dextrinizing ability are nearly identical as regards alpha-amylase activity. Malts F and H are striking in their lack of parallelism. Malt F is more active than H both as regards saccharification and dextrinization, yet it has a lower alpha-amylase activity.

In addition to the activities shown, the data of Table II obviously permit conclusions regarding the beta-amylase activity of certain of the samples. For example the fact that malt F has a higher saccharifying ability than malt H must be attributed to a greater amount of the beta component; otherwise its lower alpha-amylase content would unquestionably result in less saccharification. Likewise the observed saccharification values of malts E and F do not indicate much if any difference in their beta-amylase activities. Assuming equal amounts of beta-amylase a difference in saccharifying activities would be predicted solely on the basis of their alpha-amylase content. Thus the determination of specific alpha-amylase activity is not only valuable as such but in addition facilitates a clearer interpretation of the role of both the amylase components of malt in starch degradation.

### Summary

Wohlgemuth values as commonly determined are indicative only of the dextrinizing power of a malt extract. This dextrinization results from the combined activity of alpha- and beta-amylase. Without modification the method therefore does not offer a means of measuring relative alpha-amylase activity.

It was found that increasing the ratio of beta-amylase to alpha-amylase in the starch-enzyme mixture increased the rate of dextriniza-



tion up to a point beyond which further increments of beta-amylase have essentially no effect. The basis of a standardized technique for the quantitative measurement of alpha-amylase therefore involves the addition of sufficient supplementary beta-amylase to eliminate the variable effect of beta-amylase already present in the malt extract.

The preparation and use of an easily reproducible red-brown dextrin-iodine solution as a standard end point is described.

The linear relationship between alpha-amylase content and dextrinization time permits calculation of alpha-amylase units as the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 30° C.

Application of the method to barley malts is discussed and the lack of parallelism between alpha-amylase activity and either saccharogenic or dextrinogenic activity of malts is demonstrated.

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# A MODIFICATION OF THE WOHLGEMUTH METHOD FOR THE DETERMINATION OF ALPHA-AMYLASE AND A COMPARISON OF THIS METHOD WITH A VISCOSITY METHOD

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(Read at the Annual Meeting, May 1939)

It is generally believed that malt diastase contains at least two active components, alpha-amylase and beta-amylase. When malt diastase acts on a starch substrate the starch is first liquefied to dextrin by alpha-amylase and the dextrin thus formed is then converted to sugar, or saccharified, by beta-amylase. This dual nature of malt diastase has been made the basis of much research and several theories are advanced to explain the nature of the components of diastase. Blom, Bak, and Braae (1937) found that the liquefaction and dextrinization of starch proceed in a parallel manner, and they conclude that both are caused by alpha-amylase. This theory is supported by Józsa and Johnston (1935). Waldschmidt-Leitz and Mayer (1935), on the other hand, believe the liquefying enzyme and the dextrinizing enzyme to be two distinct enzymes. Assuming the former theory to be correct, it is entirely feasible to estimate the degree of liquefaction of a starch substrate by measuring the degree of dextrinization and *vice versa*.

This work was undertaken with the view in mind of selecting an accurate and, at the same time, a rapid method for estimating the alpha-amylase content of malt, the method selected to be suitable for routine control analysis in the malting, brewing, and distilling laboratory. Two methods were studied, a modification of Wohlgemuth's (1908) iodine-conversion method and the viscosity method of Józsa and Gore (1930) and Józsa and Johnston (1935). The modification of Wohlgemuth's method measures the alpha-amylase activity of malt by determining the time required for the malt enzyme to liquefy a starch substrate to dextrin, the end-point being determined by comparing the iodine color of the malt-starch mixture with the iodine color of a standard dextrin solution. The Józsa and Gore (1930) and Józsa and Johnston (1935) viscosity method estimates the alpha-amylase content of malt by measuring the change in viscosity of a mixture of malt extract and specially prepared raw starch solution. The two methods, although radically different from each other in mode of operation, showed satisfactory correlation with each other,

since each was found to vary to the same extent when tested with varying concentrations of the malt enzyme (Table I).

TABLE I

COMPARISON OF ALPHA-AMYLASE VALUES DETERMINED WITH THE MODIFIED WOHLGEMUTH METHOD AND WITH THE VISCOSITY METHOD OF JÓZSA, GORE, AND JOHNSTON

Sample	Enzyme concentration	Alpha-amylase value (Wohlgemuth)		Viscosity method	
		Merck's dextrin	Baker's dextrin	Starch liquefied	Liquefons per 10 cc.
				mg.	
A	Full	60.0	54.5	1986	3.259
A	Full	60.0	53.3	1991	3.282
A	Full	60.0	54.5	2031	3.456
A	Full	—	—	2031	3.456
A	Full	59.3	53.4	1904	2.929
A	Half	29.3	27.6	1096	1.024
A	Half	—	—	1103	1.033
A	Half	29.6	—	1017	0.924
A	Half	—	—	1000	0.903
Av.	Full	59.8	54.0	1989	3.276
Av.	Half	29.5	27.6	1054	0.971

Note: Sodium-chloride extraction was employed for both methods in Table I in order to obtain a true comparison of the two methods.

The modification of the Wohlgemuth method as devised by the authors combines several features of the modifications proposed by Hills and Bailey (1938) and Blish, Sandstedt, and Kneen (private communication). The method of recording the conversion values in terms of cc. of 2% starch solution liquefied is, in essence, the same as the method used by Hills and Bailey. The temperature of reaction, the use of a standard dextrin tube for color comparison, and the method of arriving at the end-point were derived from a tentative method submitted to the authors (private communication) by Blish, Sandstedt, and Kneen. The following is an outline of the authors' modification of the Wohlgemuth method:

### Reagents

*Stock iodine solution:* 11 g. iodine crystals (C.P.), 22 g. KI (C.P.), made up to 500 cc. with distilled water.

*Dilute iodine solution:* 2 cc. of stock iodine solution and 20 g. KI made up to 500 cc. with distilled water. (Made up fresh daily while malt is being extracted.)

*Standard dextrin solution:* A saturated solution of dextrin is made up as follows: 2 g. of dextrin is shaken with 100 cc. of water at 20°C.

and allowed to settle for one hour, maintaining the temperature at 20°C. throughout. One cc. of the clear supernatant liquid is pipetted into 5 cc. of dilute iodine solution in a  $\frac{1}{4}$ -inch test tube to make up the standard dextrin tube for color comparison. Baker's dextrin gives results approximately 2 minutes higher than Merck's dextrin in the 20 minute conversion range (Table I).

*Acetate buffer solution:* 68 g. sodium acetate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ) dissolved in 500 cc. of N acetic acid and the solution made up to one liter with distilled water.

*Starch solution:* 10 g. of Lintner soluble starch (dry basis) dissolved in water according to the method prescribed for the standard method of diastatic power determination (A.S.B.C.), 10 cc. of acetate buffer added and the whole made up to 500 cc. with distilled water.

### Procedure

Twenty-five grams of finely ground malt is extracted with 500 cc. of distilled water at 20°C. and filtered according to the standard method for the determination of diastatic power (A.S.B.C.).

Ten cc. of the filtered extract is diluted to 100 cc.

Twenty cc. of 2% Lintner soluble starch solution is pipetted into a 250-cc. electrolytic beaker and placed in a constant-temperature water bath at 30°C.

During the extraction period 5-cc. portions of dilute iodine solution are pipetted into each of 18 ( $\frac{1}{4}$ -inch) test tubes.

Ten cc. of the diluted malt extract, at 30°C., is added to the 20 cc. of 2% starch solution and mixed thoroughly. Timing is started with a stop watch the instant the malt extract comes into contact with the starch solution.

Ten minutes after the malt extract is added to the starch 1 cc. of the mixture is added to the first iodine tube. This procedure is repeated at appropriate intervals until the color of the dextrin tube has been passed. The color of the tubes is compared with the color of the dextrin tube and that tube which exactly matches the standard is taken as the end-point. The result is reported to the nearest one-half minute.

### Calculation of Alpha-Amylase Activity

In the method outlined above 1 cc. of 2% starch solution is acted on by 0.5 cc. of diluted (1 : 10) malt extract, or by 0.05 cc. of original malt extract. Then,  $1/0.05 = 20$  cc. of 2% starch solution is acted on by 1 cc. of original malt extract. If the conversion of the starch to dextrin takes place in  $t$  minutes, in 1 hour 1 cc. of original malt extract

will convert  $\left(\frac{60}{c} \times \frac{1}{0.05}\right)$  cc. of 2% starch solution. Formula:

$$\frac{60}{c} \times \frac{1}{0.05} = \text{alpha-amylase value.}$$

The method as outlined above is well suited to rapid control work without departing from the principle of the Wohlgemuth method (1908). It is accurate, since it is possible to obtain checks with it to within one-half minute in conversion time. It is rapid since it utilizes the same solutions and the same malt extract as used in the determination of diastatic power according to the official method of the A.S.B.C., thus eliminating the necessity of preparing special solutions and a special malt extract.

### Experimental

In order to determine the effect of beta-amylase on the alpha-amylase test, the alpha-amylase test was performed with a papain-digested extract of barley as the source of the beta-amylase. Five grams of finely ground barley were extracted for 21.5 hours at 20° C. with 50 cc. of water containing 0.5 g. of papain (Sallans and Anderson, 1938). The diastatic-power values of two different barley samples were, respectively, 323° and 350° Lintner. The two barley extracts, although very high in diastatic power, had no perceptible color effect on the iodine test tube, even after 2½ hours of action, when tested for alpha-amylase activity according to the authors' method.

Table I shows definite correlation between the two methods when the alpha-amylase value is compared with milligrams starch liquefied by the viscosity method. One-half of the true value of the malt is obtained with both methods when the malt extract is diluted to one-half its original concentration. A proportional correlation of this nature is evidence of the reliability of the two methods. However, when the alpha-amylase value and milligrams of starch liquefied are compared with "liquefons per 10 cc.," which is a measure of the actual enzyme content of the malt extract (Józsa and Johnston, 1935), the proportionality no longer holds true. No attempt is made in this paper to explain this inconsistency.

Table I also shows the difference in results with the modified Wohlgemuth method when Merck's and Baker's dextrans are used in the preparation of the standard dextrin tube. Baker's dextrin gives results consistently lower than does Merck's dextrin. To avoid confusion, results are reported only in terms of Merck's dextrin in the following tables.

Table II and Figure 1 do not show a very high degree of correlation between alpha-amylase activity and diastatic power, but they do show a general tendency toward correlation between the two values.

TABLE II

COMPARISON OF ALPHA-AMYLASE VALUES, DETERMINED WITH THE MODIFIED WOHLGEMUTH METHOD, AND DIASTATIC-POWER VALUES, EXPRESSED IN DEGREES LINTNER

(Diastatic power values arranged in ascending order.)

Malt number	Alpha-amylase value	Diastatic power
1	41.4	112.0
2	38.7	114.0
3	41.4	158.0
4	53.3	159.0
5	57.1	162.0
6a	58.5	170.0
6b ( $\frac{1}{2}$ conc.)	29.6	—
7a	61.5	174.0
7b (check)	61.5	—
8a	52.2	177.0
8b (check)	52.2	—
8c (check)	53.3	—
9a	64.9	181.0
9b (check)	64.9	—
10	58.5	183.0
11	60.0	190.0

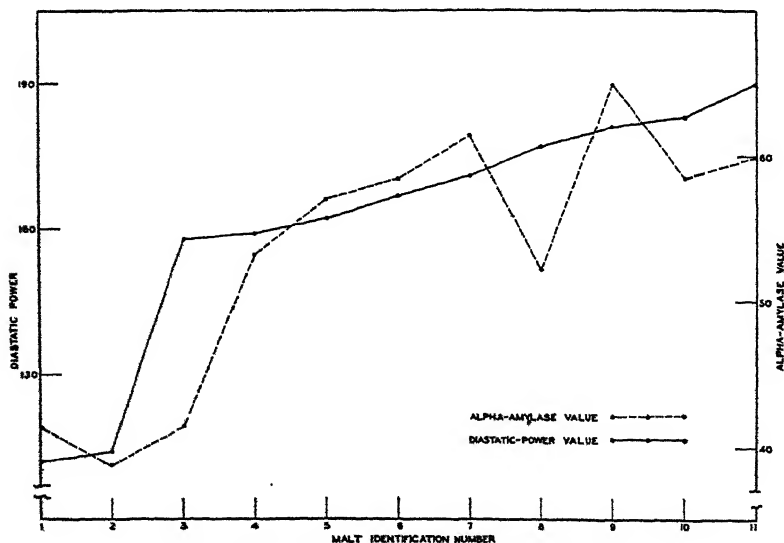


Fig. 1. Comparison of alpha-amylase and Lintner values.

According to the data in Table III the greatest activity of alpha-amylase appears to lie between 50° and 60° C. Because of lack of time only one experiment was conducted at these different temperatures. The data are included in this paper with the suggestion that further work can be done along this line with the thought in mind that perhaps better differentiation between malts will be effected at a higher temperature than at the temperature of 30° C.

TABLE III

THE EFFECT OF TEMPERATURE ON THE ALPHA-AMYLASE ACTIVITY OF MALT

Temperature	Alpha-amylase value
30°C.	55.2
40°C.	94.1
50°C.	137.1
60°C.	102.1
70°C.	Less than 30

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## THE PERMEABILITY OF BREAD BY AIR

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(Read at the Annual Meeting, May 1939)

During a study of the effect of temperature on dough properties by Baker and Mize<sup>1</sup> it was noted, as shown in Charts 9 and 10 of that paper, that there was a difference during baking in the pressure of two doughs which were identical in all respects except state of oxidation. The pressure in the oxidized dough became very much higher during baking than in the unoxidized dough and remained high to the end of the baking period. This difference in the ability of these doughs to hold gas pressure suggested that the final baked breads under these two conditions must have been different in their porosity. In other words, the one that withstood the greater pressure must have produced a bread which did not permit gas to pass through it readily, whereas the other with less resistance to pressure must have produced a bread which permitted gas to pass through it more freely.

After numerous devices had been tried to test that hypothesis, the machine shown in Figure 1 was developed. This is a device for drawing air through bread and metering the amount of air so obtained. In operation, a special blower of constant speed draws air through the sample of bread across a fixed area and passes it into an air meter of a wide range. This meter is calibrated to an arbitrary scale which can be converted into liters of air per minute if desired. The bread is subjected to increased negative pressure when a low rate of air flow takes place. This pressure is sufficient to distort slightly a thin slice of bread. The circle which determines the area of the bread through which air is drawn is bounded by a slightly elevated flat ring. The bread lying upon this ring is drawn tight by the negative pressure of the machine. If a freshly cut slice is used which has had no drying, a satisfactorily tight seal is accomplished. Also, a ring is provided which can be pressed above the seal to assure no leakage. It is necessary to make certain, in case sliced bread is used, that the particular slice being tested has no noticeable holes such as are apparent if the slice is held up to a fairly strong light. These open-textured slices should be discarded unless the device is being used to indicate the presence of holes in bread. The crust has little effect. Tests on a half loaf are more reproducible and are usually slightly higher than on slices.

<sup>1</sup>J. C. Baker and M. D. Miz Effect of temperature on dough properties, I, Cereal Chem. 16: 517-533, 1939.



For permeability readings one should select the most uniform area obtainable in a slice, preferably located in the same zone in all slices—that is, either in the top or the bottom. In our work we prefer to record the values read from the bottom half. That test which gives the least passage of air is taken as the reading and is the one most nearly duplicated on successive tests with other slices of a loaf. In

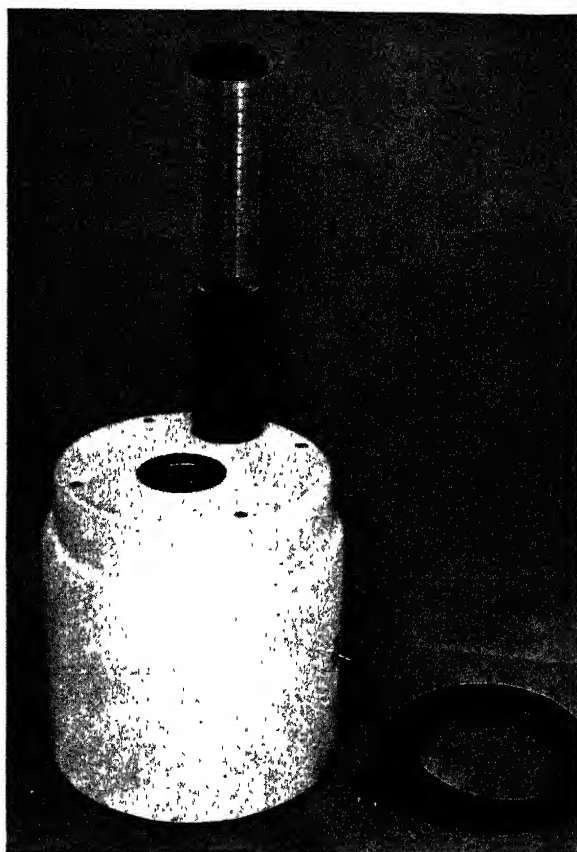


Fig. 1. Device for drawing air through bread.

other words, when testing the permeability one attempts to find how much air can be drawn through that portion of the texture which represents the normal character of the bread. In testing sliced bread not less than five pieces should be averaged.

A wide range of commercial breads, as well as laboratory breads of various composition which have been baked under a variety of conditions, have been tested in this machine.

Figure 2 gives the correlation between cell size and permeability and is determined on a large series of commercial breads by skilled observers. We are indebted to S. J. Lawellin and M. D. Mize for making the determinations on which this chart is based. It is to be noted that the permeability increase is approximately proportional to the increase in cell size. The larger the cells become the more permeable is the bread. It is also to be noted that breads which have been twisted are generally much less permeable than the untwisted bread. These results are in agreement with our previous observation that the finest-textured breads were obtained from doughs which had a high pressure during baking, whereas doughs which gave a low pressure were generally of coarse texture.

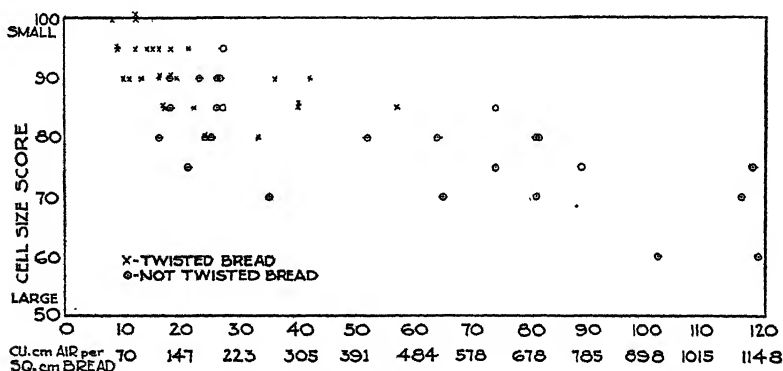


Fig. 2. Relation of permeability to cell size—commercial pan bread slices.

A study has been made of several variables in their relation to permeability. The list is not complete and much more information needs to be obtained by further work. Those factors which decrease the permeability are oxidation, fermentation, protein content, twisting of dough, and baking in a closed pan, and those which increase the permeability are semi-solid shortening and yeast and water content. It is to be noted that in general those dough conditions which improve bread quality, such as oxidation, protein content, twisted loaf, fermentation, etc., decrease the permeability of the bread, whereas factors which have a tendency to produce bread of coarser texture have also a tendency to increase the permeability.

The more permeable bread cools at a more rapid rate. A permeable slice dries rapidly; the flavor seems to disappear sooner in such bread and the crumb stales more rapidly. In the last instance it is possible that the increased staling observed is somewhat associated with the more rapid rate at which water dries out from the crumb. Fresh bread decreases in permeability, at first rapidly, then very slowly.

A study of commercial bread shows that the bread of some bakeries keeps within a fairly narrow range of permeability, whereas other commercial breads vary over a wide range of permeability. In general, those bakers who keep within a narrow range bake the less permeable bread and those whose breads vary widely bake the more permeable kind. Breads from some small bakeries are usually highly permeable, excepting those made without the use of shortening, in which case they are generally fairly impermeable even when of very coarse texture. Apparently shortening has an effect which prevents the cellular structure from finally closing or sealing the pores. This is directly opposite to the action of shortening during the earlier stages of baking, as shown in a previous paper.<sup>2</sup>

## QUALITY TESTS ON HARD RED WINTER WHEATS<sup>1</sup>

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(Read at the Annual Meeting, May 1939)

The question of the relation of protein content of wheat to baking strength and baking quality has received so much attention that it might seem superfluous to add another paper to the great number already published on this subject. However, certain modifications of methods recently used in this laboratory have led to conclusions somewhat different from those previously reported for winter wheats, and more in agreement with the commercial practice of buying on protein content.

As it is proposed to discuss estimates of both baking strength and baking quality, it is necessary at the outset to differentiate these terms clearly. The classical definition of a strong flour given by Humphries is "one capable of producing bold, well-piled loaves." This definition clearly refers to what we shall term *strength*. It involves no consideration of the differences in handling properties nor of baking procedure necessary to produce the large well-piled loaf. It should be recognized that it is possible to have flours of equal strength but of very different qualities, and *vice versa*. There has been a tendency to confuse these two baking factors, although a number of cereal chemists have stated the differences clearly. Thomas (1917) referred to strength as "that quality which enables the baker to produce a loaf of bread of large volume and of good texture by use of the proper ingredients together with the proper mixing, fermentation and baking." He recognized

<sup>2</sup> Baker and Mize, Effect of temperature on dough properties, II, Cereal Chem. 16: 682-695, 1939.

<sup>1</sup> Contribution No. 62 from the Department of Milling Industry.

that there are rather wide variations in the physical properties of doughs made from different flours. More recently Blish and Sandstedt (1935) again emphasized this point, and proposed the theory that the protein content of wheat may be regarded as the ultimate criterion of strength. They stated that owing to various characteristics of wheats it might be necessary to employ different methods for obtaining the expression of this strength. The concept of baking strength seems to be relatively simple and might be stated concisely as the capacity of flour to produce large loaves of good texture and the ability to confer this property to blends with softer wheats.

The term *quality* has so many applications and has been used so indiscriminately that it is very difficult and perhaps even unwise to attempt to define it. If the discussion of baking quality is confined to bread flours, there are clearly two legitimate applications of the term. Its most general use is in reference to the suitability of a flour for a specific bread-making process. Used in this way, there can scarcely be more than two sorts of quality, namely satisfactory and unsatisfactory. Occasionally, price consideration may induce bakers to tolerate and use flours which ordinarily would be considered unsatisfactory. For example, a typical strong hard winter baker's patent might be regarded by one baking company as of satisfactory quality, whereas another company using a procedure designed for a different type of strong flour, say a hard spring, might regard it as of poor quality, even while recognizing the existence of its high strength. Again, there is the case of high-protein flours, high in baking strength but of doubtful quality for the commercial baker's use. What baker would judge a 17% protein Turkey flour to be superior in quality for bread making to a normal 12% patent? Thus high strength may be associated with poor quality from the standpoint of suitability for a given purpose. On the other hand, low-strength flours may be deemed eminently satisfactory and hence of good quality for certain purposes such as for cakes or for pastry.

The other general application of the term *quality* is in reference to differences expressed as divergences from expectation based on protein content. The value of loaf volume to be anticipated from a consideration of the protein content is expressible as the regression coefficient or the line of regression of loaf volume on protein content, and is determined from a large number of observations. If, then, the sample having been tested by the most favorable formula available produces a volume much less than that estimated from the regression line, one must conclude either that its gluten protein is of lower bread-making potentialities than normal, or that the formulas applied were inadequate properly to express the volume of the protein. Blish and Sand-

stedt (1935) expressed this idea as follows: "When the test loaf fails to fulfill the expectations that its protein content would justify, it is likely to be suspected that the gluten was 'weak' or of inferior quality. Experience has shown however that there is every justification for challenging the baking method rather than the flour itself."

Unquestionably the possibility that the baking method is inadequate must be carefully considered. There is however little doubt that classes or varieties of wheat may differ materially in respect to the optimum baking results obtainable. This means that quality differences of the gluten proteins may exist. The question to be considered is: Shall we refer to such flours as "weak," which means the same as "low strength," or merely as of poor quality? It is perfectly obvious that *weak* and *low quality* should not be regarded as synonymous because one may have two high-protein flours both "strong" in the sense defined by Blish and Sandstedt, but one poorer in quality than the other, as shown by its failure to produce as great loaf volume as its protein content would lead one to expect. The same sort of differentiation can be observed with low-strength flours. Without laboring this point further, it might be well to consider some of the ideas that have been held concerning the relation of protein content to baking performance.

For many years it was thought that the extent to which protein could be expressed in loaf volume varied with the amount of protein present. Thomas (1917), Stockham (1920), Shollenberger (1923), and others published results of extensive baking tests which led to the conclusion that the relative effect of protein content on loaf volume is greater in the lower-protein range than in the higher. The data of Thomas (1917) and Shollenberger (1923) have been reproduced graphically in Figure 1. Such data clearly justified the conclusion stated above. Bailey and Sherwood (1926), using data obtained from the crops of 1921 to 1925 inclusive, calculated the formula of the curve which would best represent the relation between loaf volume and protein, and found it to be hyperbolic, indicating that "each increment of increase in protein content results in a diminished increment of increase in loaf volume."

Larmour (1931) reported the study of 665 samples of Canadian hard red spring wheat grown in one season. He concluded that the curvilinearity of the relation between protein and loaf volume was limited to the extreme ranges of protein, and that when these were eliminated from the series, the regression of loaf volume on protein was linear between the limits of 7.0% and 15.9% when the loaf volume obtained with the bromate baking formula was used. This conclusion seems to receive confirmation from the work of Aitken and Geddes

(1934), which showed that composites representing the range from 12.1% to 16.5% gave essentially a linear relationship between protein content of wheat and loaf volume as obtained by means of the malt-phosphate-bromate baking formula. These data are reproduced graphically in Figure 2. A high degree of correspondence exists between the two sets of data, which were obtained on crops three years apart. Larmour's data were obtained by examination of individual samples, while those of Aitken and Geddes were procured on composites made up from 7500 samples.

There seems little reason to doubt that as far as the hard spring wheats are concerned, loaf volume can be regarded as a linear function

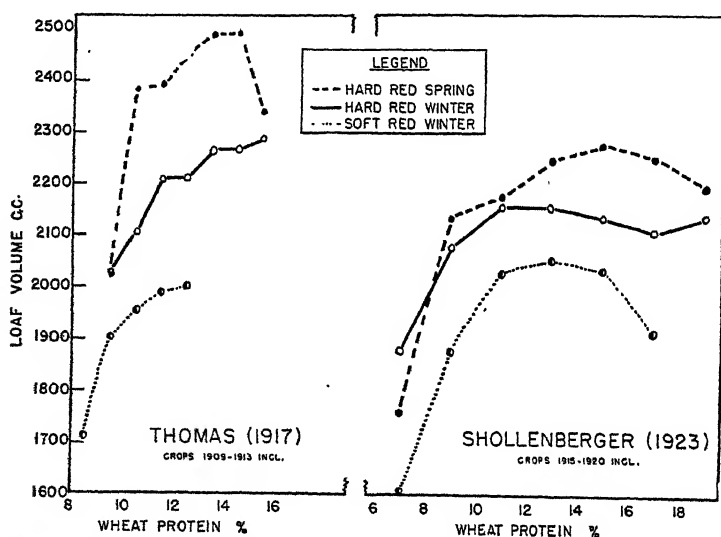


Fig. 1. The relation between loaf volume and protein content of wheat as shown by the data of Thomas (1917) and Shollenberger (1923).

of the protein content of the wheat, or flour, provided the baking is done by a suitable method.

With the hard red winter wheats, there never has been much evidence of a high degree of correlation between the loaf volume and protein content. Referring to Figure 1 again, it can be seen that although Thomas's data indicated a continuous increase in loaf volume with increasing protein content, Shollenberger's showed that beyond about 12% there was no further increase and even some indication of a decrease. Inspection of the curves for Shollenberger's data would lead to the general conclusion that the correlation between loaf volume and protein ought to be higher in the case of the hard red spring than in the case of the hard red winter wheats, because the former continued to

show progressive increases in loaf volume up to about 15%. The data of both Thomas and Shollenberger would lead to the further conclusion that beyond 12% in protein content the hard red spring wheats are indubitably higher in strength than the hard winter wheats of corresponding protein content. It is curious that Zinn's (1923) correlations showed the highest values for Kansas winter wheats,  $+ .75$ . This appears to contradict the conclusion to be drawn from Shollenberger's

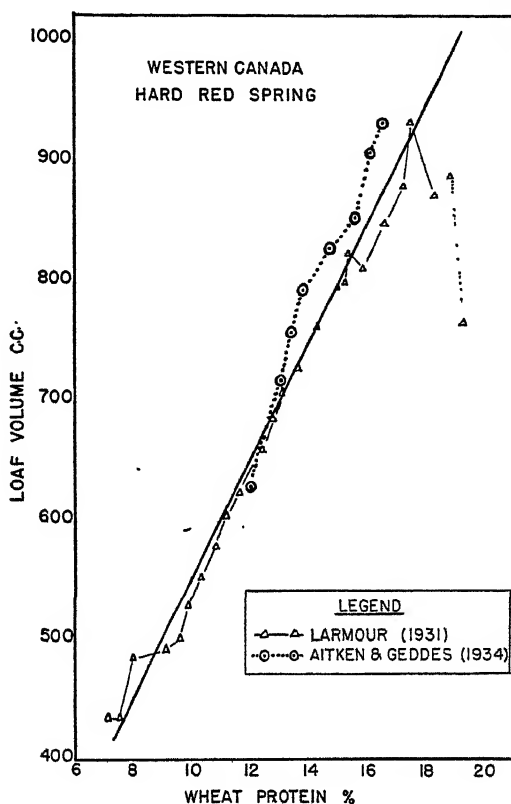


Fig. 2. The relation between loaf volume and protein content of Canadian hard red spring wheat as shown by the data of Larmour (1931) and Aitken and Geddes (1934).

data, which were based on a very large number of samples grown during the years 1915–1920 inclusive. Our recalculation of Zinn's correlation for Kansas wheats shows that it was in error; it should have been  $+ .42$  instead of  $+ .75$ . Blish and Sandstedt (1925) reported a correlation between loaf volume and protein content of  $+ .30$ , which is quite out of line with Zinn's originally reported correlation for Kansas wheats, but corresponding in magnitude to the corrected value.

It has seemed to the authors that it is practically impossible to accept as fact that there could be such a high relationship between protein and strength in hard red spring wheats and such an exceedingly low degree of correlation between these factors in hard red winter wheats. Probably failure to show a greater degree of correlation in winter wheats ought to be attributed to inadequacy of baking methods used in testing them. This view is strengthened by the observations of many investigators whose data indicate that the winter wheats of ordinary commercial protein range are fully equal in baking strength to spring-wheat flours of the same range. It would seem strange furthermore that commercial millers have continued paying premiums for high-protein wheat. Without accepting these reasons as more than indications of a probable relationship between strength and protein content, it was decided to assemble a series of samples suitable for the purpose of studying with some degree of exactitude this relationship in general, and also in its particular application to a number of the principal varieties at present being grown or in the process of being introduced in the American southwest.

### Experimental Discussion

Through the cooperation of A. L. Clapp of the Kansas Agricultural Experiment Station, it was possible to obtain pure samples of the principal varieties of wheat grown in Kansas. These samples were produced from seed supplied by the College, and were grown in 55 counties of the state. The varieties represented were Turkey, Blackhull, Kanred, Tenmarq, Cheyenne, Chiefkan, Early Blackhull, Kawvale, Clarkan, Harvest Queen, and Michigan Wonder. The first seven are classed as hard red winter wheats and the last three as soft red winter wheats; Kawvale is classed as semi-hard. In this paper only the first six varieties will be discussed. They are those of principal interest, and of greatest distribution, and the numbers in the series as finally made up were the largest.

All the individual samples were harvested under supervision, shipped to the College, and there threshed. They were then analyzed for protein and grouped on this basis. Each sample included in a composite was carefully scrutinized for damage, and a number were discarded on that account. No composite contained less than three samples, each from a different county, and many in the middle protein range contained ten or more. The well-mixed samples were milled on the Buhler mill, the flours were analyzed, aged for three weeks at room temperature, and then placed in the cold storage room at 3° C.

Material of this sort has the advantage that the varieties are known to be pure and that the whole range of protein occurring in a given



season is reasonably well represented. This is the most desirable method for comparing varieties, and is the one to be recommended whenever it is possible to obtain a sufficient number of samples. The method of comparing samples grown in one place under the same environmental conditions is commonly used, and undoubtedly possesses certain advantages, but it must be recognized that the data so obtained are not so comprehensive as when the whole protein range of the variety is studied. This seems particularly true in a consideration of qualitative differences that may be brought out by physical methods.

The flour samples were baked by two different formulas. Formula I, which was deemed the best available at the time, involved the use of the following ingredients: Flour 100%, water as required, yeast 2%, sugar 6%, shortening 3%, salt 1.5%, dry milk solids 4%, potassium bromate 0.001%,  $\text{NH}_4\text{H}_2\text{PO}_4$  0.05%, malt extract (120° L.) 0.35%. The doughs were mixed to optimum consistency, fermented, proofed, and baked according to the time schedule of the American Association of Cereal Chemists standard method using the tall, narrow pan. Punching was done by means of the National Pup Sheeting Rolls, and molding by the Thompson Laboratory Molder. Doughs were mixed with 200 g. of flour and divided immediately after mixing. Mixing was done by means of the Swanson-Working dough mixer.

This formula was decided upon in consultation with Karl F. Finney, who had been carrying on an extended investigation of various formulas suited to the requirements of hard winter wheats. While recognizing that it is difficult to obtain the information desired concerning baking strength of different varieties at different protein levels by application of a single formula, it was at the time thought that this would most probably give more information than any other single baking test. This test is comparable in many respects to the malt-phosphate-bromate test used in the Canadian laboratories. The work of Aitken and Geddes (1934) showed that with hard spring wheats this gave the greatest range and was adequate to measure the strength over a wide range of protein contents. The formula as modified for use with the winter wheats contains less yeast and more sugar, with shortening and dry milk solids additional. Finney and Barmore (1939) have advanced excellent reasons for the use of such combination of ingredients in experimental baking of hard winter wheats.

After the first baking was completed the remaining flours were stored in the cold room at 3° C. Sometime later a second baking was made using a formula which was devised as the result of observations made by Finney and Barmore (1939) and by Ofelt (1939). Finney and Barmore observed that with 4% dry milk solids in the formula and increasing increments of potassium bromate, the majority of

hard winter wheats appear to show optimum loaf volume, together with excellent crumb characteristics, with 0.004% to 0.006% potassium bromate.

Ofelt (1939) made an extensive study of the effect of increasing amounts of potassium bromate with 6% dry milk solids, using as a check a comparable series without milk. He found that while without milk the optimum bromate requirement varied considerably, with 6% dry milk solids there appeared to be a general optimum at about 0.004% potassium bromate with unbleached, experimentally milled flour. This means that a flour which ordinarily requires 0.001% or less bromate and decreases in volume with higher dosages with a formula having no milk, may in the presence of 6% milk attain its maximum volume at say 2 mg. dosage, and maintain it through 3, 4, and even 5 mg. dosages. In short, the milk in this concentration appears to create a condition in which an excess of bromate above that required for maximum development does not create the condition characteristic of overdosage. At the same time a flour requiring a high dosage can apparently make use of the bromate. The presence of 6% dry milk solids seems to establish a peculiar sort of tolerance toward bromate, so that it is possible to use sufficient for those flours requiring high dosages, without running the risk of overdosing those flours in the series that require less bromate. Some typical results from Ofelt's (1939) data are shown in Figure 3. The effect discussed above is quite evident and needs no further elaboration.

As a result of these experiments it was considered advisable to rebake the protein series, and this was done, using formula I modified as follows: 6% dry milk solids was used in place of 4%; both malt and ammonium phosphate were omitted; and 0.004% potassium bromate was used in place of 0.001%; otherwise the ingredients and conditions were the same.

One exception was made in the case of the Tenmarq variety. This is known to require considerably less bromate than the other winter-wheat varieties, and since there was material sufficient for only one baking it was deemed inadvisable to take the risk of overdosing. Consequently 0.003% was used with this variety.

The baking data by both formulas are given in Table I, and are shown graphically in Figure 4.

It can be seen from the graphs that in most instances formula I gave loaf volumes which show a relationship to protein content comparable to that shown by Thomas (1917) and Shollenberger (1923). They increased with increasing protein content to about 12% or 13% and thereafter either decreased or exhibited a very slight upward trend. Exceptions to this were observed in Tenmarq and Cheyenne, the

former showing virtually a linear relationship throughout the protein range, and the latter a maximum at 14.7%.

With formula II there was a notable change in the relationship of loaf volume to protein content. Turkey, Blackhull, Kanred, and Chiefkan gave loaf volumes which continued to increase with increasing protein content throughout the range of the latter. In the case of Kanred the highest protein sample, containing 17.7%, was 10 cc.

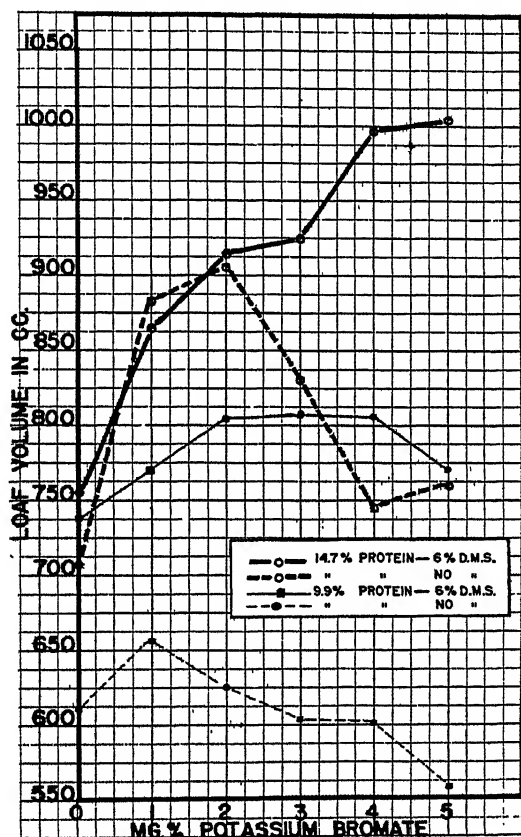


Fig. 3. The effect of dry milk solids in relation to various increments of  $\text{KBrO}_3$  in the baking formula.

lower than the one next below. This was probably due to insufficient bromate. This particular sample was rebaked with 0.006% bromate and gave a volume of 1,073 cc., which is more nearly in line with the other volumes for this variety. Attention should be directed particularly to the fact that in the lower protein ranges of these varieties the loaf volumes obtained by formula II agree well with those by formula I, which would indicate that the 4-mg. dosage had not penalized those

TABLE I  
BAKING DATA AND DESCRIPTION OF SAMPLES

Variety	Flour protein	Formula I		Formula II	
		Loaf vol.	Texture	Loaf vol.	Texture
Turkey	8.2	cc. 708	8.3	cc. 658	8.0
	9.5	708	8.3	733	8.0
	10.1	752	9.0	743	8.5
	11.0	785	9.4	798	9.5
	11.7	822	9.1	843	9.5
	13.2	812	9.4	898	9.0
	14.7	835	8.5	1003	9.0
	16.5	858	7.8	1020	7.0 open
	17.9	830	7.2	1078	7.0
Kanred	9.4	725	9.0	655	7.5
	11.0	727	9.4	770	8.5
	12.3	787	9.1	850	9.0
	13.9	822	8.8	930	8.5 open
	14.9	790	8.5	958	8.0 open
	16.2	778	8.1	1038	7.0 open
	17.7	748	7.1	1028	7.0 open
Blackhull	10.0	752	8.8	750	8.5
	11.2	775	8.5	783	10.0
	12.2	792	8.5	840	9.5
	13.8	810	7.9	863	9.0
	15.2	820	7.6	945	8.0 open
	16.3	830	7.2	1010	8.0 open
	17.6	840	6.6	1088	7.0 open
Tenmarq	8.6	730	8.6	653	8.0
	9.2	750	8.6	668	8.5
	10.1	808	9.3	750	9.5
	10.7	822	9.4	773	9.5
	12.7	908	9.4	860	9.0
	13.4	925	9.4	933	8.5
	14.9	962	9.7	975	7.5 open
	17.0	1038	8.8	1075	7.5 open
Cheyenne	8.2	702	7.9	678	8.0
	9.1	742	7.9	705	8.5
	10.1	752	7.9	725	8.5
	11.0	832	9.4	753	8.5
	12.4	840	8.7	820	9.0
	13.7	842	8.1	845	9.0
	14.7	892	8.1	920	8.5
	16.3	872	7.4	865	8.0
Chiefkan	10.4	688	8.3	690	8.0
	11.0	696	7.7	679	8.0
	12.1	710	7.4	717	9.0
	13.3	700	7.4	755	9.5
	14.6	750	7.5	800	7.5
	16.9	768	6.9	898	7.0 open

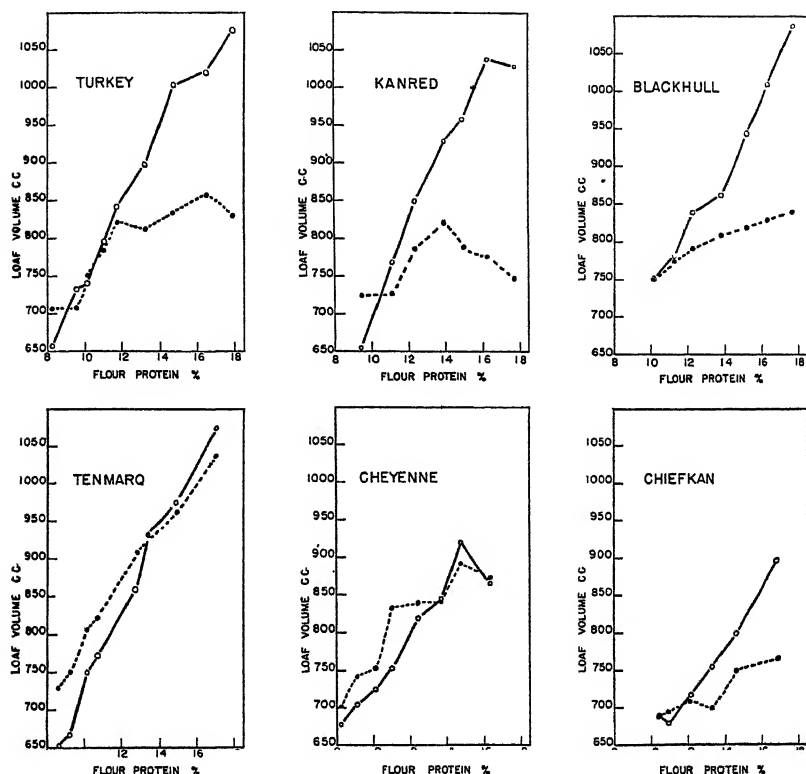


Fig. 4. The relation between loaf volume and protein content of flour as shown by two different baking formulas for six varieties of hard red winter wheat.

The broken graph line represents formula I; the unbroken line represents formula II.

samples any more than the 1-mg. dosage. It appears that the curves obtained by formula II are merely the projection of the initial portion of those obtained by formula I, which of course is in line with what one might be led to expect from theoretical considerations.

The case of Tenmarq is somewhat different from the others, inasmuch as the loaf volumes by both formulas show a linear relationship with protein content throughout the range of the latter. The difference is in the slope. It looks as though the line had been pivoted at a protein content of about 13%. This would indicate that the 3-mg. dosage depressed the lower-protein samples of the series and increased the volume of the higher ones, a result that was anticipated from Ofelt's (1939) earlier observations. Cheyenne shows evidence of similar characteristics. It has been generally thought that Cheyenne requires rather heavy dosages of bromate, but the results obtained in this study tend to cast some doubt on that conclusion. One curious result of the second baking is that the graphs for the different varieties

are so nearly the same that it is almost impossible to show them on the same figure. That is the reason for presenting them as six separate units in Figure 4.

Attention should be directed particularly to the two graphs of Chiefkan, and especially to the lower-protein samples. The three lowest samples gave essentially the same loaf volume by both formulas I and II, indicating that the additional 3 mg. of bromate used in formula II had no harmful effect. This means further that the volumes represented may be considered the greatest obtainable with any dosage of bromate. As the curve by formula II is virtually linear, it seems reasonably safe to assume that it represents the maximum loaf volume to be expected from samples of this variety at the various protein levels studied. Even if one were not prepared to accept this conclusion for the whole series of Chiefkan flours, it ought to be acceptable for the three lower-protein samples. Assuming the validity of this conclusion, it is interesting to note that the loaf-volume values for this variety are materially displaced toward the right as compared with the other five varieties shown in Figure 4.

After studying these data more carefully the authors decided to determine what might be considered the theoretical relationship between loaf volume and protein content for hard winter wheats in general. According to Quisenberry and Clark (1938) 91% of the total hard red winter wheat acreage in 1934 was represented by Turkey, Blackhull, and Kanred. It seemed reasonable therefore to use these varieties as a criterion. Loaf volume by formula II and protein content of flour give a correlation coefficient of  $+ .980$  and a regression coefficient of 43.8. The regression of loaf volume on protein content of flour, based on the data of these three varieties, is given in Figure 5. The various points lying about the line represent the individual values from which the regression was computed. There is little doubt that all these values may be considered samples of one population. In other words, it is improbable that Turkey, Blackhull, and Kanred are differentiated in respect to loaf volume at any point in their protein ranges.

The values for Tenmarq and Chiefkan are also shown in Figure 5 and are indicated by means of the connected points. It can be seen that the data for Tenmarq fit the regression practically as well as any of the standard varieties.

Chiefkan is undoubtedly an example of wheat possessing distinctly inferior quality within a class. The baking strength over its whole range of protein is markedly different from that of the typical varieties. It is not only displaced to the right but shows evidence of a lower regression coefficient, indicating that loaf-volume response to increas-

ing protein content is less than in the standard varieties. At comparable protein levels its volume is approximately 100 cc. lower than the standard. Another way of comparing them is to note that a volume of 800 cc. requires 11.2% protein for standard varieties and 14.6% for Chiefkan. There can be little doubt that this variety is sharply differentiated from the others and is distinctly inferior to them.

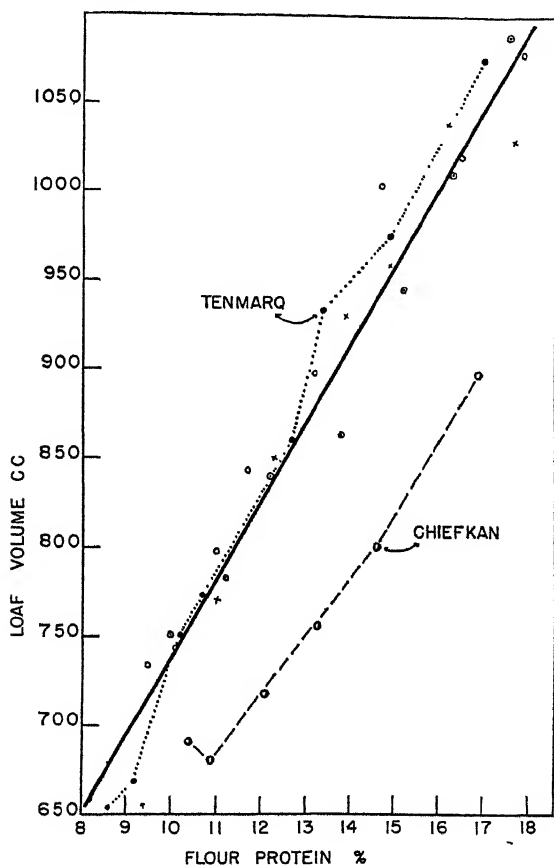


Fig. 5. The regression of loaf volume on protein content of flour.

It might be argued that the baking formula used in this instance is not adequate for the purpose of indicating the inherent strength of this variety. However reference to the data in Table I or to Figure 4 will show at once that this assumption is not tenable, at least in connection with any of the baking ingredients that are commonly used at the present time. Both formulas gave practically the same results in the lower protein range. Obviously if more bromate were required,

the graph obtained with the data from formula II ought to have been distinctly displaced upward in the low-protein range. As this does not occur it would indicate that there is sufficient bromate and that failure for the lower-range samples must be attributed to qualitative differences comparable to the sort of differences that are thought to exist between classes of wheat.

From a consideration of the results obtained with formula II, it is evident that the relationship between protein content and loaf volume is definitely linear throughout the range from 8.2% to 17.7% flour protein. This is excellent support of the contention that protein content is a measure of baking strength within the same varieties, but not always between varieties, as shown by Chiefkan. The astonishing thing is that it could be demonstrated by the application of one single formula to different varieties over a wide range of protein.

### Physical Characteristics of Doughs

For a number of years much interest has been shown in various physical methods for estimating baking strength and baking quality. Unfortunately it was not possible to make comparative tests with this series of samples because the different types of machines were not available, and the amount of flour was quite limited. The flours were examined by means of a new modification of the Swanson-Working recording dough-mixer designed by Working for use with small samples of flour. A photograph of this instrument is shown in Figure 6. The mixing principle is essentially the same as that used in the older machine described by Swanson and Working (1933) but the bowl and mixing head have been reduced in size so that 35 g. of flour is required instead of 400 g. A number of other modifications dealing with transmission of torque have been introduced. The instrument will doubtlessly undergo further refinement by the present manufacturers.

Mixing curves on all samples of the six hard red winter wheat varieties heretofore discussed are shown in Figure 7. It should be pointed out first that the curves obtained with this micro-mixer are sharper than those customarily produced on the larger machine. This is due partly to higher speed and partly to the somewhat different relationship of area of the pins in both the bowl and the mixing head. In general the small mixer produces a somewhat more pronounced differentiation between various types of flours.

It is not the purpose of this paper to attempt to interpret all the characteristics of these mixing curves in terms of baking performance of the flours, but rather to call attention to the extent to which flours may be distinguished by this means. Looking over the whole series of curves, one can see that they vary over a wide range. The highest-



protein Cheyenne sample may be taken as one extreme, and almost any of the Chiefkan samples as representing the other. The Cheyenne curve referred to has a long mixing time,  $7\frac{1}{2}$  minutes, indicating rather slow dough development; the band is broad, and continues so over a

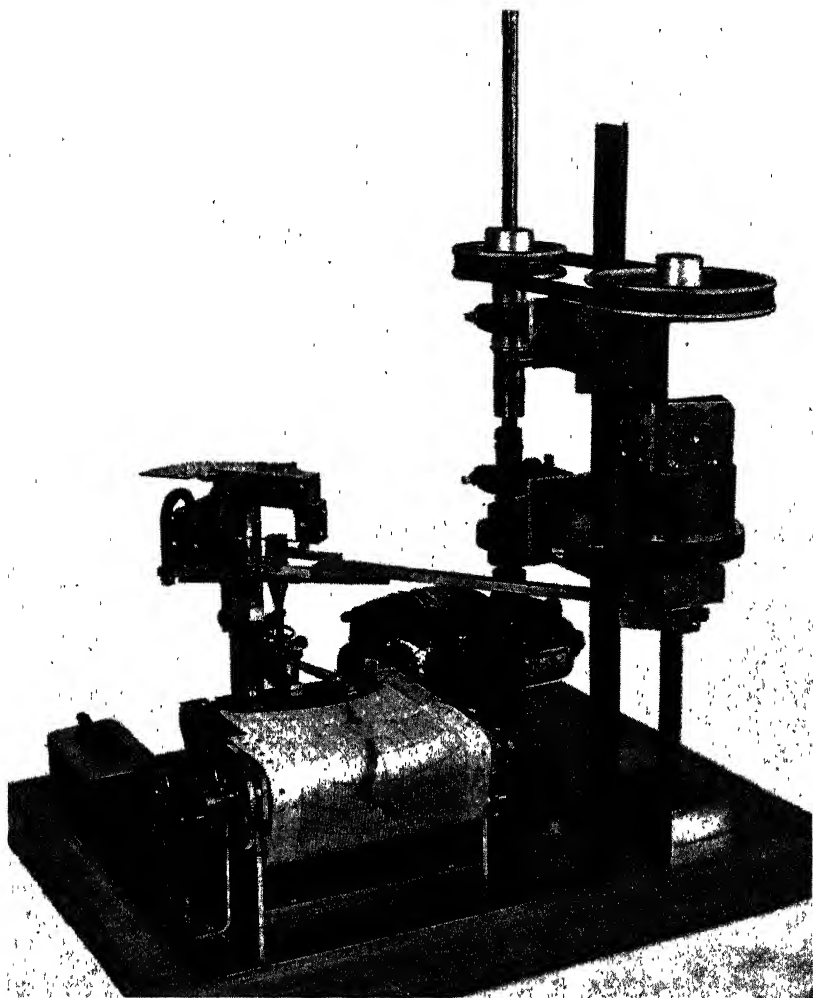


Fig. 6. The original recording micro-mixer constructed by Working.

long period of time; the rate of decline from the maximum is slow. This curve is typical of high-protein Turkey, Tenmarq, Cheyenne, and many spring wheats. The Chiefkan curves show very rapid rise to a maximum,  $1\frac{1}{2}$  minutes; the band is narrow except at the peak; the rate

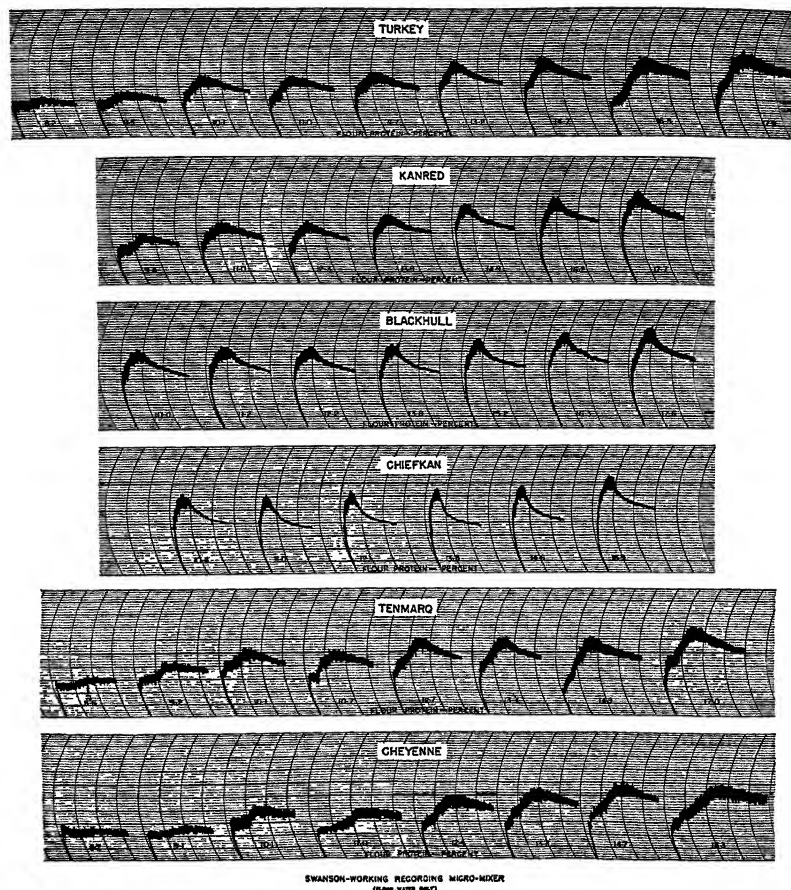


Fig. 7. Recording-micro-mixer curves for six varieties of hard red winter wheat at various protein levels.

of decline from the maximum is very rapid and the curve tapers off to an exceedingly thin line, indicating great decrease in resiliency of the dough. There is also another type of curve shown by the lowest Turkey sample. This is very flat, has considerable width, and is low. This seems to be typical of the low-protein Turkey, Tenmarq, and Cheyenne.

Perhaps the most striking thing in the series of curves is the similarity of the Blackhull and Chiefkan. These seem to be modifications of one type. The question arises, how can the differences in curve type of Turkey and Blackhull be reconciled with the fact that they appear to be of equal baking strength, and how can the similarities of Blackhull and Chiefkan be reconciled with the fact that they are

distinctly of different baking strength? The obvious answer is that the curve characteristics must be attributable to *qualitative* differences between flours. It becomes clearly evident from examination of the curves that not only do varieties differ in respect to the rate at which their doughs can be mixed to the maximum resistance consistency, but they also differ markedly in the manner in which they behave after this maximum has been passed. The curves thus give information not only concerning the mixing requirement but also concerning their behavior during prolonged mixing. Although they are similar in type it is possible to distinguish between Chiefkan and Blackhull because the former requires consistently shorter mixing time and breaks down more rapidly and more completely than the latter.

Regarding the difference between Turkey and Blackhull, the curves show a marked *qualitative* distinction among varieties in respect to behavior. This has been recognized for a long time by the commercial bakers. The baking tests heretofore described have shown these varieties to be equal in strength; the curves show them to be different *qualitatively*.

Tenmarq and Cheyenne appear to be similar to Turkey in dough characteristics and equal to it in baking strength with the exception of the highest-protein Cheyenne sample. Unfortunately there is not sufficient information available to decide whether or not Tenmarq is qualitatively different from Turkey. It was baked with 0.003% bromate in place of 0.004% and thus it is impossible to say that the two varieties are the same from the standpoint of the treatment required to bring out the maximum baking strength. It is currently believed, however, that Tenmarq requires less bromate than Turkey. From that standpoint it may be said to be qualitatively differentiated from Turkey, but there is no definite evidence in the data herein presented to support this condition.

While certain differences between some varieties are very obvious, it can be seen that within some of the varieties rather marked differences in shape of the curves exist. This applies particularly to Tenmarq, Kanred, Turkey, and Cheyenne. The curves in the low-protein samples tend to be low and flat; as the protein increases, they become sharper and higher. In these varieties the height of the curve appears to be fairly closely related to the protein content, and thus may be taken as an indication of strength. In contrast to these four varieties, Blackhull and Chiefkan exhibit a remarkable uniformity of curve type throughout the protein range. The range is less than in the case of the other varieties and one cannot state definitely that they would flatten out in the case of low-protein samples, but there is little evidence of any such tendency. Until further data are available it

seems justifiable to conclude that the curve type of these varieties is fairly uniform and persists through any range of protein content that might be encountered. Some evidence in support of this belief is to be found in the curves made with Clarkan, a soft red winter wheat selection from Blackhull. Clarkan at 9% protein gives curves somewhat similar to the Chiefkan curves. It seems safe to state that this particular curve type is a persistent and uniform characteristic of the Blackhull group of wheats, and it may be used with confidence to distinguish them from the Turkey types, with the possible exception of the higher-protein Kanred samples.

It may well be asked what use can be made of curves of this sort when so much variation exists, and particularly when such varieties as Blackhull and Turkey, both of equal strength, show such different curve characteristics, and furthermore how could one distinguish Chiefkan? In the first place, from the commercial point of view, it is highly desirable that millers should know the kind of wheat they are using. When Blackhull was introduced, it caused a great deal of trouble, mainly because bakers did not know how to use it. At present it is widely recognized as a useful wheat and complaints about flour produced from it have practically disappeared. Millers and bakers, if convinced of the strength of a wheat, can undoubtedly discover how to use it to advantage. But in order to do so they must gain some knowledge concerning its characteristics, and that involves being able to distinguish it. There is no doubt that anyone familiar with the curve characteristics of a variety such as Chiefkan could easily distinguish it by means of its mixing curve, principally on the basis of its exceedingly short development time and its very marked decrease in resiliency after the maximum has been passed. Unlike Blackhull, however, this selection differs from Turkey on two qualitative scores, namely its physical dough characteristics and the inferior quality of its protein as shown by the baking test.

### Summary and Conclusions

The data presented in this study support the conclusion that within a given season the potential strength of the principal hard red winter wheat varieties is related to protein content in linear fashion, and is very highly correlated with it. It has also been shown possible to obtain this expression of baking strength by means of a single formula applied under one set of fixed conditions. The formula involves the use of 2% yeast, 6% sugar, 6% dry milk solids, 0.004% potassium bromate, and 3% shortening with mixing to optimum consistency and the standard fermentation and proof times of the American Association of Cereal Chemists. When this method was used with composite samples

of Turkey, Kanred, and Blackhull, the correlation between loaf volume and protein of flour was  $+ .98$ . This indicates that all but 4% of the variability of loaf volume was accounted for by variation in the protein content of the flour.

When 6% dry milk solids is used in the formula, it is possible to include sufficient potassium bromate to condition the flours of the highest bromate requirements without overdosing those of very much lower requirement. While the function of the milk is not clearly understood, it seems evident that it creates a rather broad tolerance toward bromate, thus permitting the use of much higher increments of bromate than would otherwise be possible.

Mixing curves obtained by use of the Swanson-Working instrument reveal certain marked distinctions between varieties within the hard winter wheat class as well as within varieties in the class. Varieties such as Chiefkan exhibit such distinctive curve characteristics that they are recognizable at all protein levels.

In the opinion of the authors, the greatest usefulness of the mixing curves, provided the protein content of the flour is known, is to characterize the type to which the flour belongs. They serve to establish *qualitative* differences between wheats that may or may not be equal in strength, and thus give an indication of the manner in which they may be expected to perform. In many instances it is possible to make fairly good estimates of baking characteristics from a consideration of the curve. For instance the low, broad, flat curves shown for the lower-protein range of Turkey are particularly characteristic of low-protein hard wheats, both winter and spring. Soft winter wheats of corresponding protein content give a quite different type of curve and one that can be readily recognized. Again, the high, broad curves of the upper protein levels of Turkey are characteristic of the strongest sample of hard winter wheats and very similar curves are obtained with high-protein samples of standard varieties of hard spring wheats. Such curves are therefore interpreted as indicating the highest strength as found in the hard types of wheat.

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## STUDY OF CHECKING AND pH IN CRACKER AND BISCUIT PRODUCTS

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(Read at the Annual Meeting, May 1939)

The first indication that crackers and cookies "check" is the appearance of very fine cracks (similar to those observed in products manufactured by the cement, ceramic, and steel industries), hardly noticeable at first but later causing the goods to break and fall apart. Checking must not be confused with tenderness. Too tender goods may break easily like the checked goods; however, they have good chewing and eating qualities, whereas the checked goods, being brittle and tough, are deficient in these qualities. In crackers this condition is often described as "chipsy," "chipping off," and "flinty."

Checking is treacherous because it may appear at the time of packing and then again not until the goods have reached the merchant or consumer. In some cases, such as in army rations or when the goods are shipped to distant points, checking may not disclose itself for many months. In any event, the checked crackers and cookies

will not stand ordinary handling and considerable breakage results in transportation.

Dunn and Bailey (1928) show several very fine photographs of checked cookies, and although their work has been of great assistance to the industry, checking is still something of a mystery despite extraordinary precautions taken in the production because it seems to appear and disappear, apparently without any reason, and particularly so under certain seasonal and climatic conditions.

The two main factors associated with checking are internal stress and brittleness. The product may resist this internal stress for many months but finally will crack because within these forces a contraction usually predominates, resulting in shrinkage. The hypothesis explaining this condition is as follows:

We may assume the flour to be the most important ingredient, and call the cracker and cookie dough a complex colloidal matter, or we may assume the other ingredients such as water, fat, etc. as more important than the flour, and call the dough (in a broad sense) an emulsion, and a dry emulsion if baked. However, we believe that checking is associated with the flour condition and that we are dealing with a highly complex colloid. If we study the matter from this standpoint, we may divide the goods made by the cracker and biscuit industry into two groups:

(1) Stable colloids—goods with no internal stress, which do not check under any conditions, nor does any other change take place.

(2) Unstable colloids—goods with internal stress. Depending upon the degree of this stress, they will check immediately after baking, during cooling, or within a few days, weeks, or months.

In working on this problem, I utilized knowledge gained in studying ceramics under Kalauner at Brno—that is, the behavior of the soil and clay (a colloidal matter) by the addition of very dilute acid and alkali. If very dilute alkali is added, the particles do not separate but stay in the suspension, whereas the dilute acid separates slowly into two distinctive phases. One is the almost clear water on the top and the other is the sediment on the bottom. In the manufacture of crackers and cookies the case is similar because of the fact that we deal also with a colloidal matter and that the chemicals<sup>1</sup> are added, bringing the dough to either a slightly alkaline or acid reaction. For this reason, the pH value was studied and it was found that this was one of the basic causes of checking.

<sup>1</sup> In the cracker and biscuit industry, the term "chemicals" includes sodium bicarbonate (soda), ammonium bicarbonate (ammonia) and various acid bases all used as leavening agents.

## Lean and Semi-lean Formulas

First, checking was studied in the goods made with a very lean formula consisting of flour and water alone with very little or no enriching ingredients, and particular attention was paid to the type of machine used in the manufacturing. It can be seen from Table I that hard breads or hard tack, water crackers, and pilot breads are made from flour and hot water mixed into a very stiff dough, and with no chemicals added they usually check because they are on the acid side as a result of the natural acidity present in the flour. Checking occurs irrespective of whether soft-winter or a blend of soft-winter and hard-spring wheat flours is used. Pilot breads, containing little enriching ingredients and only a small amount of chemicals, check as long as the chemicals are so balanced that they will bring the pH of the baked product on the acid side.

TABLE I

INGREDIENTS, pH, CONDITION OF CHECKING IN HARD-TACK, PILOT BREAD, SPRAYED, AND LUNCH MILK CRACKERS

Flour	Water	Sugar	Fat	Salt	Soda	Tartaric acid	pH	Remarks
HARD-TACK								
100	35	—	—	—	—	—	5.8	Checking
100	52	—	—	—	—	—	6.0	Checking— made from spring-wheat flour
PILOT BREAD								
100	35	4	7	1	0.25	0.125	6.4	Checking
100	34	4	7½	1½	0.1875	0.21875	5.8	Checking
100	34	3½	7	2	0.375	—	7.2	No checking
100	36	5	7½	½	0.50	0.25	6.6	Checking— blend with 6% of spring wheat flour
SPRAYED CRACKER								
100	26	5	15	1	0.50	0.50	6.0	Checking
100	26	5	15	1	0.625	0.50	6.4	Checking
100	26	5	15	1	0.75	0.50	6.8	Checking
100	26	5	15	1	0.75	0.375	7.2	No checking
LUNCH MILK CRACKER <sup>1</sup>								
100	25	3½	15	1	0.625	0.50	6.4	Checking
100	25	3½	15	1	0.75	0.375	7.2	No checking

<sup>1</sup> In the lunch milk cracker, water is replaced by whole or skim milk.

A sprayed cracker is similar to pilot bread but rich in fat. The extra added fat reduces the quantity of added water. In comparing the formulas listed in Table I, it can be seen that using two ounces less of tartaric acid (the difference between 0.50 and 0.375) per 100



pounds of flour, or 4 ounces per 200 pounds of flour, changes the pH from the acid to alkaline side. In the alkaline medium, no checking or very little was observed. Several years ago this cracker was made without the spraying of cocoanut oil and it used to check very badly if the pH was on the acid side. Spraying with cocoanut oil has a stabilizing effect, but there is always the danger of checking if the cracker is on the acid side. The other factor influencing the checking in this product is that it is made on the cutting machine in such a way that after cutting about one-third of the dough is returned and added to the fresh dough. This procedure is repeated over and over again and this, of course, is very detrimental to the stability because although this dough is otherwise perfect, it has been subjected to the machining many times. This of course may account for some checking even if the product is on the alkaline side.

All the baked products listed in Table I were made from unfermented doughs, being mixed with hot water so that the temperature of the doughs ranged from 85° to 115° F., and in machining they were usually put twice or oftener through the upright brake rolls, then through the sheeting rolls on the cutting machine, and later cut. If certain precautions are taken in mixing, machining (for instance thorough perforations in cutting), and in baking, even though the product is on the acid side it may not show signs of checking for a long time.

### Soda Crackers

As can be seen from Table II, acid soda crackers usually checked, but not always. In this cracker an insufficient amount of soda was added in the dough stage and consequently the baked cracker was on the acid side. The dough lacked elasticity and plasticity, did not run smoothly on the machine, and became short and tough. The baked cracker took on color too quickly, having an unnatural burnt color, lacked oven spring, and was tough and brittle ("chipsy" or "flinty"). However, even the crackers on the alkaline side may check if the flours are mistreated in mixing, in fermentation (over-ripe or young sponge, or too stiff or too soft sponge, or hot sponge and dough), in machining and baking. Crackers made from an over-ripe sponge check more than those made from young sponge.

Improper blending of flours is another cause of checking of crackers on the alkaline side—that is, if the flour blend is too soft or too strong, or if too soft or too strong flours are blended together. The crackers made from a blend of hard-spring and soft-winter flour will check even if on the alkaline side. In fermentation, hard-spring-wheat flours do not mature under the same conditions as those made from soft-

TABLE II  
EFFECT OF pH AND FERMENTATION ON CHECKING IN SODA CRACKERS

Soda crackers	Fermentation time, in hours		pH	Remarks
	Sponge	Dough		
Alkaline	19	4	7.2	No checking
"	18	5	7.8	No checking
Acid	19	4	6.0	Checking
"	18	3	6.4	Checking
"	23	5	6.6	Checking
"	19	5	6.6	Checking
"	20	5	6.6	Checking
"	19	4	6.8	Checking
"	19	5	6.8	No checking
"	18	5	6.8	No checking
Over-ripe sponge	23	5	7.4	Checking
"	23	4	7.0	Checking
"	23	4	8.0	Checking
"	22	4	7.6	Checking
Young sponge	15	5	7.4	Checking
"	16	4	7.2	Checking
Too soft sponge	18	4	7.6	Checking
Too stiff sponge	20	5	7.4	Checking
"Hot sponge"	18	4	7.2	Checking
"	19	3	8.0	Checking
One-hour proofing	20	1	7.8	Checking
Flour blend too soft	20	3	7.2	Checking
Flour blend too strong	20	4	7.4	Checking
Flour blend with 10% spring-wheat flour	Not known	Not known	8.2	Checking
Flour blend with 20% spring-wheat flour	Not known	Not known	7.6	Checking
Flour blend with 40% spring-wheat flour	Not known	Not known	8.6	Checking

winter wheat and crackers made of such a blend lack in fermentation flavor, have no oven spring, and are heavy. In cracker baking, blending of the hard-spring and soft-winter wheat flours is rare; however, this happens once in a while in Canada if the flour is extremely soft and where the supply of soft-winter-wheat flour is limited.

Poor baking is responsible for checking of the cracker on the alkaline side. If the heat in the oven is so balanced that the cracker does not spring as soon as it is placed on the hot oven shelf, the cracker will not rise at all during the latter part of baking. When baked it is solid and heavy. Furthermore, even a perfectly made soda cracker with pH on the alkaline side may develop a little checking because as much as 10% of the dough used has already passed machining (the

two outside strips beyond the cutting head, which are added to the fresh dough and very often not evenly distributed). This operation is very detrimental to the stability of the cracker doughs as well as the baked cracker.

In machining the soda-cracker dough passes twice through the upright brake rolls, then the sheeting rolls and the cutter on the cutting machine. In this manner the dough may be abused to a certain extent.

Cheese crackers (soda cracker with added cheese) are usually on the acid side with as low a pH as 5.0 and since cheese has a stabilizing effect, as has also the cocoanut oil which is used for spraying, there is little checking. However, under certain conditions checking may develop.

### Graham Crackers

Graham crackers (listed in Table III) are not true crackers in the pure sense of the word. They are made from an unfermented dough with soda and ammonia as the leavening agents. They lean in richness toward the cookie side. Graham crackers do not check because a sufficient amount of chemicals is added so that they are decidedly on the alkaline side, and they also contain moisture-retaining substances such as honey, molasses, etc. Besides these crackers are usually baked in large sheets the size of the peel directly on the oven shelf, whereas sweet cookies are baked individually on the pans. In machining, the dough is treated in a manner similar to that used with soda crackers.

In studying the checking of cookies according to the type of machine on which they are made, the cookies may be divided into three groups: (1) wire-cut or drop and bar cookies, (2) short-bread cookies, and (3) cookies made on the cutting machine.

### Wire-Cut and Bar Cookies

Wire-cut and bar cookies are made from very soft (flowing consistency) or semi-soft dough with short mixing for wire-cut cookies (until clear) and slightly longer mixing for bar cookies (little over clear). They are mixed very cool, with the temperature close to 70° F. In machining the dough is placed between the two revolving rolls and pressed through the die, and then, in the case of wire-cut cookies, cut by wire, whereas bar cookies are placed on the moving apron and cut by the knife. Because the flour in wire-cut and bar cookie doughs is not abused in mixing and machining and only fresh dough is used (no returned dough which already has passed the machine operation) and because a large amount of water is used in

TABLE III  
TYPE OF COOKIE MACHINE, INGREDIENTS, pH, AND CONDITION OF CHECKING

Type of machine	Kind of cookie <sup>1</sup>	Flour	Water	Sugar	Fat	Salt	Soda	Ammonia	Tartaric acid	Acid phosphate base	pH	Remarks
Wire-cut	Vanilla wafers	100	42-55	60-80	20-40	1-2	0.50-1.25	0.50-1.00	0	0	7.2-8.4	Very soft dough, no checking
"	Wafer	100	40	70	30	1	0	1.0	0	0	6.0	Acid dough, no checking
"	Jumble	100	22	50	25	1½	0.625	0	0	0.125	7.8	Semi-soft dough, no checking
Bar	Bar	100	23	45	30	1½	0	0	0	0.125	5.6	Plain acid bar, no checking
"	Cocoa nut bars	100	16-18	45-55	15-20	1-2	0.50-1.0	0.375-0.25	0	0	7.0-8.0	Plain, cocoanut 25-50, no checking
"	"	100	10-14	40-45	15-20	1-2	1.0-1.5	0.25-0.375	0	0	7.2-8.2	Molasses 15-30, no checking, cocoanut 25-50
Cutting	Tea biscuit	100	14	35-45	15-20	1	0.375	0.1875	0	0	7.0	Mixed cool at 75°F., no checking
"	"	100	14	15-25	12-15	1	0.625	0.25	0	0	7.8	Mixed hot at 90°F., checking
"	Arrowroot	100	14	40	15	1	0.25	0.125	0	0	6.6	Mixed cool at 75°F., arrowroot 5-10, checking
"	"	100	14	40	15	1	0.4375	0.1875	0	0	7.2	Mixed cool at 75°F., arrowroot 5-10, no checking
"	"	100	15	30	17½	2	0.50	0	0	0	7.4	Mixed hot at 90°F., arrowroot 5-10, checking

TABLE III—Continued

Type of machine	Kind of cookie <sup>1</sup>	Flour	Water	Sugar	Fat	Salt	Soda	Ammonia	Tartaric acid	Acid phosphate base	pH	Remarks
Cutting	Chocolate tea biscuit	100	16	40-45	17-20	1	2.125	0	0.25	0	7.8(E) <sup>2</sup>	Cocoa powder 10, no checking
"	Chocolate tea biscuit	100	16	40-45	17-20	1	2.125	0	0.25	0	7.8(E)	Cocoa powder 7½, chocolate liquor 5, checking
"	Base cake	100	14	35-40	17-20	1	0.75	0.375	0	0.375	7.8	Invert syrup 5-20, no checking
"	"	100	14	35	17	1	0.50	0	0	0.50	6.6	Acid, invert syrup 5-20, checking
"	Chocolate base cake	100	16	40-45	20	1	2.25	0	0.25	0	8.0(E)	Cocoa powder 10, no checking
"	Snap	100	15	40-55	15	1	0.75	0.50	0	0	8.0	No checking
Rotary	Short-breads	100	5-8	25-45	20-35	1-2	0.50-1.0	0.125-0.50	0	0.25-0.50	7.0-8.0	No checking
"	Chocolate short-bread	100	6-10	25-45	20-35	1	1.0	0	0	0.375	7.4(E)	Cocoa powder 5, chocolate liquor 5, no checking
"	Chocolate short-bread	100	6-10	25-45	20-35	1	1.0	0	0	0.375	7.4(E)	Cocoa powder 7½, chocolate liquor 10, checking
Cutting	Graham crackers	100	16-24	20-25	10-15	1-2	0.75-1.50	0.25-1.0	0	0	7.2-8.6	Honey and molasses 10-20, no checking

<sup>1</sup> Eggs may be used in the amount of 2 to 15 parts per 100 parts of flour in all except graham crackers.<sup>2</sup> Electrometric.

mixing (Table III), the doughs are stabilized to such an extent that even if the cookies are on the acid side, the detrimental effect of the acid is more than counteracted and the baked cookies (being spongy in grain and porous) do not check regardless of whether they are on the acid or alkaline side.

### Short-Bread Cookies

Short-bread cookies are made on the rotary machine and are usually very rich, with 20 to 35 parts of fat and with 25 to 45 parts of sugar per 100 parts of flour. Short-bread doughs are mixed for a long time at a very low speed (20 r.p.m. or lower) and so little water is added (5 to 8 parts per 100 parts of flour) that the dough is very dry and stiff and has the consistency of a paste. If the temperature is kept cool (close to 80° F.), the dough is not abused in mixing. In machining, the dough is placed between the two revolving rolls, pressed into the cups of one of the rolls, and released on the apron by another suction roll. In this operation the dough is abused very little or not at all, only fresh dough is used, and the amount of fat used is high; for these reasons the short-bread cookies do not check if they are on the alkaline side. It can be seen from Table III that chocolate short-bread cookies, made with cocoa powder and also with the combination of cocoa powder and chocolate liquor, do not check as do those made on the cutting machine. However, if the amount of chocolate liquor in the formula is too high even the short-bread pieces will check.

### Cookies Made on the Cutting Machine

Sugar cookies, hard, and semi-hard sweets made on the cutting machine, such as tea and arrowroot biscuits, base cakes, and snaps, show considerable checking. In studying the manufacturing, we can see that there are many factors in mixing and machining favoring checking. Mixing is carried on too far (overclear) and the dough becomes tough and is able to take up the returned dough which has already passed machining. The amount of this returned dough is sometimes more than the fresh dough, that is, about two-thirds in small pieces with a count per pound of 100 to 150 and about one-third in the larger pieces with a count per pound of 50. In machining, the dough passes through one to four sheeting rolls and then is cut. After cutting, the dough lying between the cookies and that on the edges beyond the cutting head is returned and mixed with the fresh dough. It can be seen that mixing and especially machining produce a very unfavorable condition for the stability of the dough and for that

reason many cookies made on the cutting machine will check even though they are on the alkaline side.

### Baking and Cooling

Checking due to improper baking may occur if too hot an oven or "flash heat" is causing excessive rising and little spreading ("poor") in the goods, or too cool an oven or lack of heat is causing excessive spreading and little rising ("rich"). It can be seen that from the same dough three kinds of cookies can be baked: that is, on the "poor" and "rich" side and with the correct shape and weight.

Checking may be due also to underbaking caused by the present high-speed production with fast baking, and in rare cases it is due to overbaking caused by baking too long a time at low temperatures (resulting in more drying than baking).

On the whole, pH has a profound effect on baking. At the present time with high-speed production, baking is carried out only to the certain desirable color. In baking the acid products, especially with pH 6.0 or lower, take up color so quickly that to prevent burning the products must be taken out from the oven underbaked, whereas the alkaline products with pH 8.0 or higher also take up color quickly with a thick crust formation but not so fast as the acid products. To assure thorough baking, both acid and alkaline products should be baked at lower temperatures for a longer time. There is no doubt that both acid and alkali affect the caramelization of the starch and sugar at baking temperatures, causing the goods to take up color so rapidly that they appear well baked when they are actually raw and underbaked. In baking, best results are obtained with pH at 7.0 (neutral) or slightly on the alkaline side with pH below 8.0, because then the product may be thoroughly baked, and the color will be derived chiefly from the ingredients used in the formula—thus the undesirable coloring effect of the acid and alkali is eliminated.

To prevent chilling, which causes checking, during cooling, two methods are employed: (1) Fast cooling so that the goods are packed hot, which condition is favorable for sogginess and rancidity. Also, cooling is accomplished in such a short time that checking has not as yet taken place but may occur later in the package. (2) Gradual or slow cooling so that the goods are packed warm but not hot. This, if properly done, should give much better results than fast cooling not only from the checking but also from the quality standpoint.

### Formulas

Checking may occur (1) if the flour used is too soft, too strong, or a blend of too soft and too strong and if no attention is paid to the

changes caused by the new crop flours; (2) if not enough or too much sugar is used, causing the goods to be on the "poor" or the "rich" side, particularly so when on the "poor" side; (3) if the chemicals act in the oven in such a way that the action of "raising" and "spreading" is not balanced.

Even with well balanced formulas, the chocolate cookies made on the cutting machine with cocoa powder or with the combination of cocoa powder and chocolate liquor show a great tendency to check even if high on the alkaline side. It can be seen that cocoa powder and especially chocolate liquor produce conditions very unfavorable for the stability of the dough.

### Effect of the Acids

An acid medium has a very unfavorable effect on dough development, causing it to become too tough with a great deal of decrease of elasticity and plasticity. The effect of acids may be disastrous if acid-reacting ingredients such as unneutralized invert syrup, honey, and molasses are mixed with flour before the soda is added, which is usually sifted last on top of the flour. In such a case the dough may become so tough that it does not retain the round shape stamped by the cutter but is deformed into an oblong shape. The round and uniform shape is very important in base-cake cookies made for sandwich and marshmallow pieces. The pH of such deformed cookies may be on the alkaline side because a sufficient amount of soda was added to bring it to this point, but the damage was done in mixing prior to the addition of the soda. In such a case, soda is not able to restore the dough to its natural condition but is at least able to prevent checking. Naturally, the dough not containing invert syrup, etc., may become too tough in mixing if chemicals are not well balanced and the pH is brought to the acid side. The toughness is caused by the acid portion of the chemicals and as can be seen from Table III such cookies check.

### General Remarks

Glycerine, invert syrup, and invert syrup containing ingredients such as honey and molasses, used as preventive measures against checking, have a stabilizing effect. Spraying with cocoanut oil has a similar effect.

Because goods on the acid side are brittle, easily broken, and check readily, a shortometer has very little value for determining the shortness of this type of product. In the products on the alkaline side, the shortometer may be of value if the tested samples have, or are brought to, the same pH value, provided they do not check.



Bohn, Gilner, and Kinder (1936) have shown that the alkalies are very detrimental to flavors such as pure vanilla extract, lemon oil, etc., and to conserve these flavors the pH should be kept on the acid side. On the other hand, our work has shown the detrimental effect of the acids, and it can be seen that the only compromise possible is to keep the pH at 7.0 (neutral) or very close to it. Soda has a very beneficial effect on the elasticity and plasticity of the doughs, if used in quantities to bring the pH slightly on the alkaline side (below 8.0). In the past this practice was overdone to a great extent and larger quantities of soda were used, bringing the pH as high as 9.0. With a pH over 8.0 not only are most of the added flavors destroyed but the product has a soapy aftertaste, probably due to a slight soap formation at baking temperatures by the reaction of soda and fat.

The pH determinations were made by the colorimetric method, and only in the chocolate pieces by the electrometric method. The colorimetric method is used extensively for checking production in the cracker and biscuit industry and appears to give better results because it shows well the excessive amount of soda used in the formula, giving slightly higher results.

### Summary

Checking in cracker and biscuit products is associated with the flour condition and is caused by internal stress and brittleness.

The governing factor is pH, not only in checking but in the whole process of manufacturing, that is, in mixing, machining, and baking, as well as in the quality of the finished product.

A pH on the acid side is responsible for checking and for the unstable condition of the doughs, causing them to become tough with a decrease of elasticity and plasticity.

A pH slightly on the alkaline side is responsible for the increase of elasticity and plasticity of the dough, causing easier machining with no checking in the finished product. However, if the flour is mis-treated in mixing, fermentation, and machining, or if there is improper baking (particularly underbaking), cooling, or an unbalanced formula, checking may occur.

In soda crackers, a blend of soft-winter and hard-spring wheat always causes checking.

Returned dough, cocoa powder, and especially chocolate liquor have a very unfavorable effect on the stability of the doughs, causing checking even if the product is on the alkaline side.

High absorption and high shortening content have a favorable effect on the stability of the doughs with a tendency to prevent checking.

In order to maintain the quality of the finished product, to insure smooth and easy machining, and to prevent checking, the pH should be kept close to 7.0 or slightly on the alkaline side.

The shortometer cannot be used for measuring the shortening value of the crackers and cookies if they are checking.

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## STANDARDIZATION OF THE SCORING OF TEST CAKES<sup>1</sup>

OLOF E. STAMBERG<sup>2</sup>

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(Read at the Annual Meeting, May 1939)

Previous sub-committees on Methods of Testing Cake Flours have expressed the need of a more systematic and standardized method of scoring the test cakes made by the present tentative A.A.C.C. method. The chairman for the 1938-39 committee assigned to the author, as a member of the committee, the task of preparing some photographic standards for the grain scores. The work was carried somewhat further, resulting in some recommendations for a standardized system for other cake scores. The series of pictures finally selected represent the composite opinion of all the committee members, and other scoring methods suggested in this report are presented here with the committee's approval.

The methods of scoring which will be presented apply only to cakes made by the present tentative A.A.C.C. formula as described in *Cereal Laboratory Methods*. The scoring system according to the present A.A.C.C. method includes the following characteristics and points for perfect scores:

A—External		B—Internal	
Symmetry	10	Texture	
Volume	15	Tenderness	15
Crust	5	Silkiness	15
		Grain	25
		Color	15

The cake scores which tell much about the flour are those on volume, symmetry, and grain. These scores can be standardized

<sup>1</sup> Paper No. 1719, Journal Series, Minnesota Agricultural Experiment Station, St. Paul. Subcommittee report, 1938-39 Committee on Methods of Testing Soft Wheat.

<sup>2</sup> American Dry Milk Institute Research Associate. The author wishes to acknowledge the co-operation of the American Dry Milk Institute, Chicago, Illinois, in allowing time for this project.

quite satisfactorily, while scores such as those on tenderness and silkiness must be based on the operator's personal judgment.

### Volume Score

The perfect volume score is 15 according to the A.A.C.C. scoring system. Previous committees on collaborative testing of cake flours with the A.A.C.C. formula have shown no uniformity in assigning this score. Last year, one operator gave a score of 5 for a cake volume of 790 cc.; another gave a score of 16, one better than perfect, for 804 cc.; and another 8 for 833 cc., and so on. Instead of such irregularity in assigning the volume score, it would be much better to use a systematic scale.

There are several methods which at first appear to be logical as bases for a volume scale. First, the score could be based on the specific volume of the cake, but previous committee work has shown that the baking loss varies considerably in different laboratories, and would thus produce differences in the specific volumes. Second, the volume increase of the batter could be used, but this would require the determination of the specific volume of the batter, and the values obtained would have little meaning. Therefore the actual volume of the cake in cubic centimeters as a basis for the score is by far the simplest and the best method and involves only one value and no calculations.

The standard test requires the use of 325 g. of batter, and with the best of cake flours now available and with the present test formula, the cake volume will seldom exceed 940 cc., but cakes with volumes from 925 to 940 cc. have been obtained. After consideration of numerous factors in connection with the volume score, the following scale was decided upon by the committee for use with 325 g. of batter:

Cake Volume (cc.)	Score	Cake Volume (cc.)	Score
940-920	15	780-760	7
920-900	14	760-740	6
900-880	13	740-720	5
880-860	12	720-700	4
860-840	11	700-680	3
840-820	10	680-660	2
820-800	9	660-640	1
800-780	8	<640	0

Some laboratories probably prefer using 350 or 375 g. of batter, but proportional scales can easily be calculated since within these limits the cake volume is practically proportional to the batter weight. This system of scoring the volume should be much better than the previously used random method, although cake volumes obtained vary as much as 100 cc. for test cakes made in different laboratories and from the same ingredients. This variation appears to be due primarily to the technique used, which should be further standardized.

### Grain Score

Numerous actual-size pictures were made of the interior of cakes and circulated among committee members, and the series of pictures finally selected represent the composite opinions of the committee members. Figure 1 shows a smaller reproduction of the actual-size

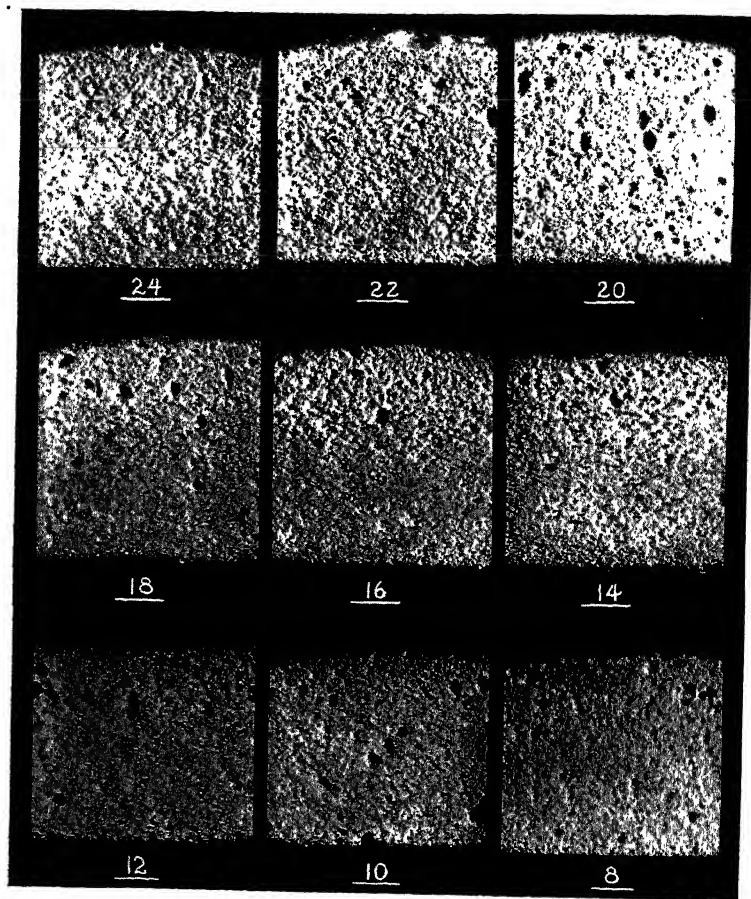


Fig. 1. Reproduction of the photographic grain standards.

picture series. The perfect grain score is 25, but it is quite difficult to produce the perfect grain, and hence that was left for extrapolation, and the best grain pictured was given the score of 24. As the scores go down from 24 to 22 and so on the cell walls become increasingly thicker and the grain coarser. Any cake which appears to match one of the standards except that it has more holes or channels can be assigned the next-lower odd score. These pictures should help some-

what in standardizing the grain scoring and should especially be an aid to inexperienced operators.

### Symmetry or Shape Score

The symmetry score actually refers to the shape of the cake as observed after it has been cut lengthwise. A high top or very convex cake will be scored low, although the cake may be perfectly symmetrical, and this score should more properly be referred to as shape.

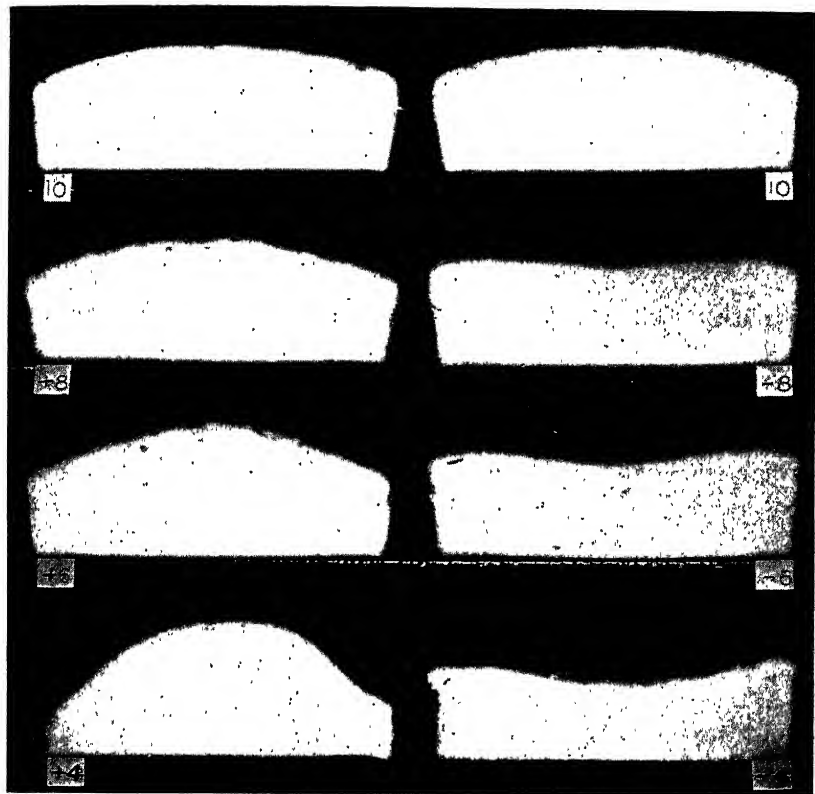


Fig. 2. Standards for the scoring of symmetry or shape of the test cakes.

This score has also been based entirely on the operator's opinion and without any standard guide. The shape of the cake is quite indicative of the cake-flour quality. A stronger-protein flour will give a more rounded convex top, while a weaker flour or a flour with low sugar or shortening tolerance will give a flat or concave top.

Figure 2 shows a series of test cakes cut lengthwise and with different shapes. The two top cakes have been given the perfect score of 10, and they have slightly rounded tops with only slight breaks.

They appear to be the shape of cake obtained by good cake flours with the present formula. It is further suggested that a simple plus or minus sign precede the numerical score on the score card for convex and concave cakes, respectively. While the extreme of one type is as detrimental as the other type, the numerical scores may be the same but the type of symmetry or shape would be indicated by the plus or minus sign. The concave cakes are especially obtained when supplements *A* and *B* of the A.A.C.C. formula are used, namely the sugar and shortening tolerance tests, respectively. The odd scores are left for interpolation of cakes which appear to come between the pictured standards.

With these possibilities of standardizing the volume, grain, and symmetry or shape scores, it is suggested that scores for such characteristics as tenderness and silkiness be varied as little as possible as long as they are based entirely on personal judgment. The volume, grain, and shape scores alone will probably classify cake flours with the formula now used, and the scoring would be more uniform in different laboratories and by different operators.

It must again be emphasized that the presented scoring methods are for cakes made by the present tentative A.A.C.C. method only, and will not apply to cakes made from other formulas. This is particularly true for the grain standards and the volume scale. If in the future the test formula should be changed, new standards could easily be worked out involving the same principles.

#### Acknowledgment

The author is highly indebted to the committee members, F. J. Coughlin, R. W. Mitchell, H. W. Putnam, W. E. Stokes, D. Wade, E. P. Walker, L. D. Whiting, and the chairman J. W. Montzheimer, for their cooperation in selecting the photographs and their advice throughout, and also to L. Armstrong, L. H. Bailey, E. G. Bayfield, P. Logue, and O. P. Skaer for their interest and advice at the committee meeting.

#### Photographs

A set of the two photographs for grain and shape can be purchased during years 1939 and 1940 for 75 cents from the Photographic Laboratory, University of Minnesota Farm Campus, St. Paul, Minnesota.

# STARCH AS A FACTOR IN DOUGH FORMATION<sup>1</sup>

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(Read at the Annual Meeting, May 1939)

Starch constitutes the major part of wheat flours and during recent years has been subjected to considerable study in relation to flour quality, enzyme susceptibility, and bread staling. Wheat-starch granules vary in size and Grewe and Bailey (1927) grouped the granules as small, medium, and large and determined the percentages by number of these groups in 17 flours. They concluded that there was no relation between the size distribution of the granules and the baking results. Buchanan and Naudain (1923) grouped the granules as below or above 7 microns, and from a study of seven flours concluded that strong flours have the largest percentage of small granules, but they considered the average size more important. They did not give the protein content of the flours used, and they based their conclusions regarding flour strength on loaf volume and grain. Naudain (1925) from a study of four flours found the baking results were poorer in the flours with a higher percentage of large starch granules.

Some studies of flour strength have been made by diluting flours with starch. Jago (1911) added 20 parts of potato, wheat, or maize starch to 80 parts of flour and observed that with added starch the baking results were poorer, and that the absorption was greater for the maize mixture than for the potato mixture. Johnson and Bailey (1925) found that the addition of starch to flour reduced the gas retention of the dough, but the gas production was not impaired. Alsberg (1935) suggested that starch may also play an important role in the variability of absorption of flours, and Pulkki (1938) found that a reduction of the flour-particle size increased the absorption appreciably, as a result of an increase of injured starch granules.

Markley (1938) diluted flours with wheat starch to various protein levels and studied the absorption necessary to produce doughs with a minimum mobility of 550 farinograph units. At about 7% to 8% protein (13.5% moisture) the minimum absorption was observed and the mixed flour-starch blends below this protein level had the physical characteristics of a starch paste rather than a dough. Markley suggested that with less than 7% protein there was not enough protein or gluten to cover the surface of the starch granules and hence the mixed blends below this protein level had the characteristics of starch pastes. The work reported here includes some further studies of the

<sup>1</sup> Paper No. 1720, Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul.

relationship of starch surface area to absorption and protein films in doughs.

### Starch-Granule Distribution in Flours

With the data of Grewe and Bailey (1927) available on the distribution of various sizes of starch granules in 17 flours, it is possible to calculate quite accurately the starch surface areas of these flours. They measured the starch granules under a microscope on the ruled hemocytometer slide and classified the granules as those with a diameter of less than 7.4 microns, those from 7.4 to 14.8 microns, and those of a diameter greater than 14.8 microns. About 700 granules from each flour were measured, and the percentage by number of each size group was determined. After some observations of wheat-starch granules under the microscope, it was decided, for purposes of calculation, to use an average diameter of 4.7 microns for the small granules, 10.1 microns for the medium-size granules, and for the large granules an average diameter of 24.9 microns. A value of 1.5 was used as the density of starch. Actually wheat-starch granules approach the shape of oblate spheroids, but for the calculations a spherical shape was assumed, which should give fairly accurate values.

In Table I the calculated surface areas in square centimeters per gram of starch are given for the 17 flours. The relative starch surface areas of the flours are also given in percentages based on the average surface area. The types of wheat used as the source of the flours are also included, as given by Grewe and Bailey, and their percentages by number of the small granules. There is a close relationship between the percentage of small granules and the surface area, which of course would be expected. The flours used by Grewe and Bailey were mainly high patent flours and different results might be obtained with other grades.

The variation in surface area was from 88.8% of flour No. 3 to 116.7% of flour No. 14, as based on the average surface area of the 17 flours, taken as 100%. Twelve of the flours were within the 12% variation or from 94% to 106% of the average, with the remaining five flours falling outside this range. The possible significance of this variation in surface area will be discussed subsequently.

Figure 1 shows graphically the distribution of the three sizes of starch granules according to number, weight, and surface area per unit weight. The values were based on the average of the 17 flours. It is evident that the large-granule fraction predominates as far as weight and surface area are concerned, and that the large granules constitute the major portion of the wheat starch and also account for most of the surface area of the starch in wheat flours. One gram of small granules with an average diameter of 4.7 microns would have



TABLE I  
STARCH SURFACE AREAS OF VARIOUS FLOURS<sup>1</sup>

Wheat used for flours	Percent by number of small starch granules	Calculated	
		Starch surface area in cm <sup>2</sup> /g of starch	Percent relative starch surface area based on average value
1. Pacific soft white	80.45	1943	97.0
2. Canadian spring	86.36	2079	103.6
3. Hard spring	59.97	1780	88.8
4. Hard winter	75.03	1912	95.4
5. Canadian hard spring	85.92	2018	100.7
6. Hard red winter	79.84	1908	95.2
7. Ohio soft winter	76.65	1802	89.9
8. Indiana soft winter	88.16	2106	105.1
9. Minnesota hard spring	77.94	1904	95.0
10. Minnesota hard spring	79.07	1915	95.6
11. Minnesota hard spring	83.01	2053	102.4
12. Minnesota hard spring	89.55	2220	110.8
13. Ohio red winter	87.78	2160	107.8
14. Nebraska hard spring	91.79	2339	116.7
15. Missouri soft winter	82.80	1965	98.1
16. Montana hard winter	74.39	1840	91.8
17. Durum	87.77	2120	105.8
Average	—	2004	100.0

<sup>1</sup> Calculated from data by Grewe and Bailey (1927).

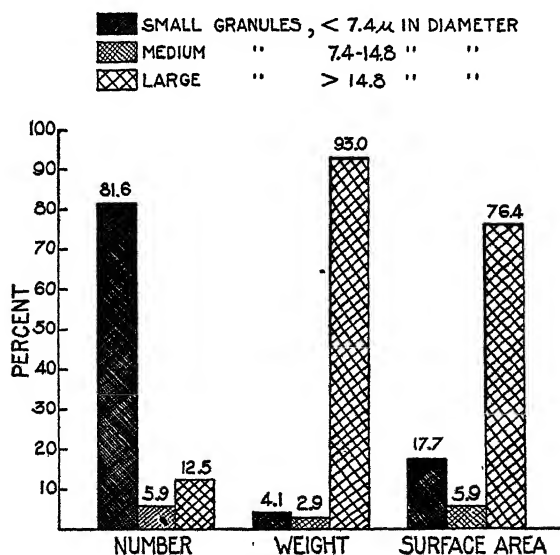


Fig. 1. The distribution of the small, medium, and large starch granules in flours according to percentages by number, weight, and surface area, and based on the average values of seventeen flours.

a surface area of 8,519 cm.<sup>2</sup> per gram, and the large granules with an average diameter of 24.9 microns a surface area of 1,606 cm.<sup>2</sup> per gram.

### Absorption of Flour-Starch Mixtures

Markley (1938) found that on diluting flour with wheat starch a minimum of the absorption curve occurred at about 7% to 8% protein, with a change from a dough to a starch-paste system below this protein level. If this change were due to an increase of the total starch surface area to the extent that the protein content becomes too low to form a continuous film or reticulum, it would be expected that by using starches of various sizes this change would occur at other protein levels. To test this assumption a series of absorption tests were made by diluting a flour with rice, corn, wheat, and potato starches, and also finely pulverized wheat starch.

A flour with 11.14% protein (15% moisture) was used. Flour-starch blends were mixed in the farinograph and the absorption adjusted to give a minimum mobility of 500 units. Two percent salt was used in all doughs. The absorption values obtained were on the basis of 15% moisture, and the amount of starch added is indicated by the protein content of the mixture.

Figure 2 shows the absorption curves obtained by diluting the flour with the various starches. Rice starch, having the smallest granules, produced a minimum absorption at about 10% protein, corn starch at 8.5% protein, wheat starch at 7.5% protein, and the larger potato-starch granules at 4.3% protein. Thus the minima of the absorption curves are in the same relative order as the size of starch granules used, indicating a relationship between starch surface area and the observed minima. Addition of wheat starch which had been ground to fragments in a rod mill increased the absorption considerably above that of the unground wheat starch, and broken starch granules undoubtedly play an important role in the absorption of flours.

### Starch Surface Area in Relation to Protein Content

To study further the relationship of the starch surface area to the absorption and to the protein content, the surface areas of the flour starch and the starch samples used were determined by measuring the granules under a microscope and then calculating the surface areas per gram by the method previously explained.

The rice-starch granules were from 4 to 8 microns in diameter and within a fairly uniform size range. The corn-starch granules varied from 8 to 18 microns in diameter. The potato-starch granules showed a wide size distribution and were classified in groups of below

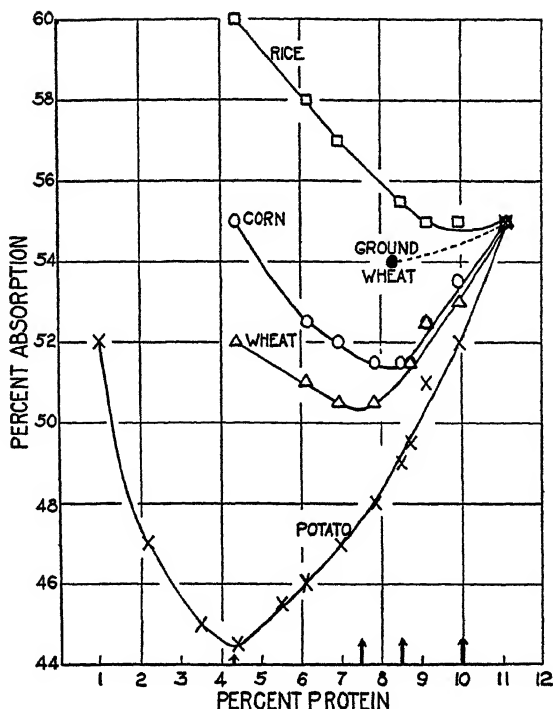


Fig. 2. The relationship of the absorption necessary to give doughs of a minimum mobility of 500 farinograph units to the protein content, upon dilution of a flour with various starches.

25 microns, 25 to 50 microns, and above 50 microns in diameter. The calculated surface areas per gram of starch are given in Table II. The approximate ratios of the surface areas per unit weight of the starches were 1, 2, 3, and 8 for potato, wheat, corn, and rice, respectively.

With the starch surface areas per gram known for the flour and the starches, it was possible to calculate the change in surface area as the flour was diluted with the starches. Figure 3 shows the change in starch surface area in square centimeters per 100 grams of flour-starch blends (15% moisture) in relation to the protein content.

In Figure 3 the slight increase in surface area upon dilution with wheat starch was due mainly to the increase in the amount of starch per 100 grams. With the other starches the change in surface area was primarily due to granule size. The potato starch produced an immediate rapid decrease in surface area, while the corn and the rice starches increased the surface considerably.

From the graph in Figure 3, the starch surface areas can be obtained at the protein levels corresponding to the minima of the absorption

TABLE II  
STARCH SURFACE AREAS

Starch	Calculated total surface area of the starch samples  <i>cm<sup>2</sup>/g</i>	Approximate ratios of total surface area per unit weight
Flour starch	1974	
Potato starch	853	1
Wheat starch	1907	2
Corn starch	3077	3
Rice starch	8000	8

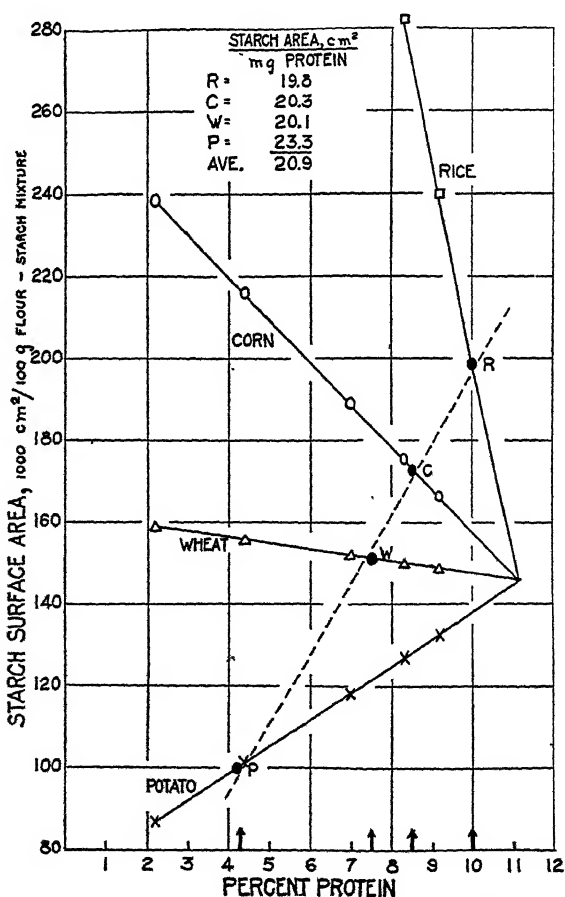


Fig. 3. Total starch surface area per 100 grams of flour-starch mixtures (15% moisture) at the various protein levels. The arrows along the abscissa are at the points corresponding to the minima of the absorption curves in Figure 2.

curves in Figure 2, namely at 4.3, 7.5, 8.5, and 10.0% protein respectively for the potato, wheat, corn, and rice starch curves. These points are shown at *R*, *C*, *W*, and *P*, in Figure 3. The ratios of starch surface area in square centimeters per milligram of protein ( $\text{cm.}^2/\text{mg.}$ ) at points *R*, *C*, *W*, and *P* were found to be 19.8, 20.3, 20.1, and 23.3, respectively, with an average value of 20.9. These values check so closely that they indicate quite definitely that in flour-water doughs mixed to the stage of minimum mobility the protein film envelops each individual starch granule. If this were not so, the ratios obtained by the various starches should not be constant since they were obtained on the basis of starch surface area.

The hydrogen-ion concentration is an important factor in studies of proteins, and the flour used had a pH of 5.74. With mixtures of half flour and half starch the pH values were 5.00, 5.72, 5.72, and 6.28 for rice, corn, wheat, and potato starch, respectively. The variations in pH of the rice and the potato starch may account to some extent for the greater deviation of the  $\text{cm.}^2/\text{mg.}$  ratios as compared to those of corn and wheat starch. Furthermore, the potato-starch granules were rather irregular in shape, and it was quite difficult to estimate the mean diameter.

The results indicate that in accordance with Markley (1938) the protein content of the flour must be over 7.5% for dough formation. With 7.5% protein at 15% moisture the amount of starch would be about 75% or on a dry basis the ratio of protein to starch must be greater than 0.1 for dough formation. There still remains a possibility that this cannot be taken as an absolute value under all conditions. If a different mixer were used with a more severe mechanical action than the farinograph, the possibility is that the break in the absorption curve would come at a higher protein level or at a thicker protein film.

### Starch as a Factor in Absorption

The data in Table I show that most of the flours included were within a 12% range of variation in surface area. Through the difference in absorption at the same protein level of any two absorption curves in Figure 2 and with the corresponding difference in surface area available from Figure 3, it was possible to estimate that with a 12% difference in starch surface area there would be a difference of 0.9%, or about 1%, in the absorption. Since the maximum variation in starch surface area of the 17 flours studied was as high as 27%, it appears that a variation in absorption due to surface area may be 2% but in the majority of cases probably is 1% or less. In addition to the starch surface area, the number of broken starch granules in a

flour is an important factor related to the absorption, as can be seen by the addition of ground wheat starch. This is in agreement with Pulkki (1938) that overgrinding of flours increased the absorption as a result of broken starch granules.

The absorption so far discussed has been in relation to a constant minimum mobility of 500 units in the farinograph. The absorptions required for best baking results with flours of various protein contents are probably different, according to Merritt and Bailey (1939), whose results indicated that low-protein flours gave best baking results at lower minimum mobilities than 500 farinograph units and high-protein flours at higher mobilities than 500 units.

A low-protein flour mixed to 500 farinograph units will feel firmer to the operator than a high-protein flour mixed to the same consistency in the farinograph. An instrument which will measure this firmness of the dough will probably give a better indication of the proper absorption required for best baking results. Here again the starch plays an important role since this firmness of doughs made from low-protein flours is undoubtedly due to the thinner gluten films separating the relatively hard starch granules. In the farinograph this firmness is apparently not distinguished from the softer but more tenacious doughs of high gluten contents.

### The Protein Film in Doughs

From the data and the discussions in the previous paragraphs it appears that, in doughs mixed to the stage of minimum mobility from flours with 7.5% protein or more, the protein reticulum envelops each individual starch granule. Assuming the proteins in a dough as a uniform anhydrous film around the starch granules, and with the average spreading value of the protein established as 20.9 cm.<sup>2</sup> per milligram at the 7.5% protein level of the flour, it is possible to calculate the thickness of such an assumed anhydrous film. With the density of anhydrous proteins as 1.3 (Neurath and Bull, 1938) the thickness of this film was calculated as 3,684Å. Much of the work on monomolecular protein films has been with egg albumin, but Neurath and Bull (1938) in a review article quote data for gliadin as having a monomolecular film thickness of 4.5Å at zero pressure and about 15Å at high pressures, and the thicknesses of various other monomolecular protein films are in the neighborhood of 10 to 12Å. Hence the assumed anhydrous protein film around the starch granules in a dough made from a 7.5% protein flour would be equivalent to about 350 monomolecular protein layers thick, and the thickness of the film between two starch granules would be about twice that amount. As the protein content of the flour increases above 7.5%

this assumed film thickness will increase proportionally. Actually the hydrated proteins or the gluten film in a dough is much thicker and is extended into a much larger volume than that of the assumed anhydrous film, and hence the values arrived at are only speculations.

There is no direct evidence, however, that in a dough the starch granules are surrounded by successive orderly arranged layers of monomolecular protein films, and a more disorganized or brush-heap structure of the protein micelles has often been discussed. The fact seems to be that on mixing flour and water into a dough a rearrangement of the proteins around the starch granules takes place concomitantly with a hydration of the proteins until the stage of minimum mobility of the dough is reached. When this stage is reached the dough will perform most satisfactorily from the standpoint of baking results. Stamberg and Bailey (1938) showed that the amount of work in watt hours necessary to complete this process of mixing and hydration increased with the protein content, and in the farinograph the required mixing time was longer with higher percentages of protein.

Some additional work has been done on protein films in milk doughs and the indications are that milk proteins will supplement to some extent the flour proteins in forming a protein film or gluten in a dough, but since it makes the picture of protein films more complex it will not be included in this discussion.

### Starch and Flour Strength .

Flour strength is a term which is used in relation to many flour factors such as protein quantity and quality, absorption, diastatic activity, and the tolerance to mixing and fermentation processes, with the ultimate criterion being the quality of bread produced. Starch plays an important role in many of these factors. The differences in enzyme susceptibility of wheat starches and the effect of these differences on diastasis and fermentation have been studied by many investigators. In relation to absorption the variation in starch-granule size must be considered, as well as the number of injured starch granules.

The starch-granule size and surface area seem to be of importance in relation to the protein film in a dough and hence probably to flour strength. Buchanan and Naudain (1923) and Naudain (1925), using loaf volume and grain as the criteria, concluded that strong flours had the largest percentage of small granules, as the result of a study with just a few flours. Grewe and Bailey (1927) in a study of 17 flours concluded that the starch-granule size variations could not be correlated with any of the baking test results. Spaeth (1915) concluded that the percentages of small starch granules were higher in

weak flours, and that the quantity of small granules depended upon soil and climatic conditions.

Let us suppose that two flours have the same protein content and one of them has a higher than average percentage of small granules, then in a dough made from the latter the protein films would be thinner and might be expected to simulate the film from a lower-protein flour with average-size starch granules. With this reasoning, it seems that a large percentage of small granules would result in a poorer baking quality, with all other factors equal. On the other hand, a high percentage of small granules would give a closer packing of the starch granules with a finer structure of the dough. During the baking process in the oven the small granules will resist swelling and gelatinization until a higher temperature is reached (Naudain, 1925; Alsberg, 1926) and the dough would have a longer period for oven expansion due to the slower gelation of the starch and setting of the dough. These and perhaps many other opposing factors are involved and it is difficult to postulate the variation in baking quality of flours due to starch-granule size variations.

Further work appears justified on the starch-granule size distribution in flours of different varieties and from wheats grown under different conditions. It is not possible with the information now available to state definitely that starch-granule size variations are of importance to any factor other than the absorption of flours, but baking quality and the rate of bread staling may be related to the granule size distribution.

### Summary

The surface area per gram of wheat starch based on the average of 17 flours was calculated as 2,004 cm.<sup>2</sup> The flours showed a variation of surface area of from 88.8% to 116.7% of the average surface area, but twelve of the flours were within a 12% range of variation or within 94% to 106% of the average.

In terms of numbers the small starch granules, below 7 microns in diameter, represent 81.6%, but in terms of weight or total surface area the large granules, above 14.8 microns in diameter, are by far the most important and represent 93.0% by weight and 76.4% of the total surface area.

The surface areas per gram of commercially prepared potato, wheat, corn, and rice starches were found to be 853, 1,907, 3,077, and 8,000 cm.<sup>2</sup>, respectively, or in approximate ratios of 1, 2, 3, and 8, respectively.

Upon dilution of a flour with the various sizes of starch granules, the absorptions necessary to produce doughs of a minimum mobility



of 500 farinograph units at various protein levels were determined. The minima of the resulting absorption curves were at different protein levels when the various starches were used. By calculating the starch surface area per milligram of protein at these minima the values were 19.8, 20.3, 20.1 and 23.3 cm.<sup>2</sup>, for the rice, corn, wheat, and potato starch curves, respectively, with an average value of 20.9. Thus the starch surface area was found to be the factor involved at the minima of the absorption curves.

It was concluded that for dough formation in the farinograph mixer the protein content of a flour must be over 7.5% (15% moisture), or on a dry basis the ratio of flour protein to starch must be greater than 0.1.

It was calculated that the variation in starch surface area, as far as the 17 flours studied disclosed, can effect a variation in the absorption of as high as 2%, but with the majority of the flours this would be 1% or less. Injured starch granules were observed to increase the absorption appreciably and may be of most importance.

Some estimations of the thickness of protein films in doughs were made on the basis of the thickness of monomolecular protein films, and in doughs made from 7.5% protein flour, the minimum for dough formation, the protein film was estimated to be some several hundred protein molecules thick, with the thickness of this film increasing with a higher protein content of the flour.

Further work is suggested on the possibility of a relationship of flour strength and baking quality to the starch-granule size distribution in flours of various grades and from different sources, since the information now available is incomplete and conflicting.

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## STARCH IN RELATION TO SOME BAKING PROPERTIES OF FLOUR<sup>1</sup>

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In general the idea prevails that the baking properties of a flour are determined chiefly by the quantity and characteristics of its gluten and that possible differences in the starch are of minor consequence. Alsberg (1935) stated that "with fuller knowledge, it may appear that variations in the properties of the starches of different flours influence baking quality materially." In flour it is exceedingly difficult to differentiate those baking characteristics which belong with the starch from those which belong with the gluten, since any treatment applied to the flour might conceivably affect either or both.

The addition by Morea (1937) and by Aitken and Geddes (1938, 1939) of crude gluten to a dough to increase its protein content and thus enhance its baking properties suggested the possibility that gluten and starch might be combined to form a "synthetic" dough which could reflect the individual properties either of the starch or of the gluten from which it was made. A synthetic dough of this type could afford a basis for the study of differences in some of the individual constituents of flour and for a study of their properties in relation to baking behavior. It might also provide an opportunity to study the effects of various flour treatments on these individual constituents and the effect of the treatment on the baked loaf.

The first attempts at combining gluten and starch in a synthetic dough were made with wet crude gluten and commercial wheat starch, but this combination baked into a very inferior loaf of bread. This suggested a possibility that either the water-soluble constituents (lost in washing the gluten) were essential to the production of a good loaf

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of bread, or that the commercial starch had been altered by some procedure in its preparation.

Accordingly fresh wheat starch was prepared by centrifuging the water suspension of starch obtained in washing gluten. Two distinct layers of material separate from the liquid in the centrifuge tube. Figure 1 shows centrifuge tubes containing the starchy material from 50-g. portions of different flours. The lower layer is relatively pure starch which contains about 35% of water and on the dry basis 0.3% of protein and 0.1% of ether extractable lipid. The upper gelatinous or semi-liquid layer seems to be the impure, highly hydrated "dextrins" resulting from the action of the flour amylases on the "available" starch. It contains 80% to 90% of water and on the dry basis, along

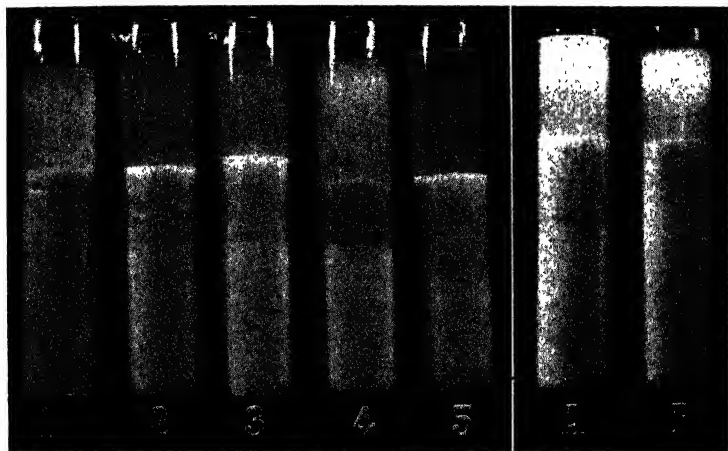


Fig. 1. (Nos. 1 to 5 at left.) Starch and amylopectin fractions from different flours.  
Fig. 2. (A and B at right.) Starch and amylopectin from flour and middlings.

with some unidentified material, about 3% of protein ( $N \times 5.7$ ) and 0.5% of ether soluble lipid. The characteristics of this "dextrin" material are quite similar to those of the "amylopectin" material which remains after the action of beta-amylase on boiled wheat starch (or on wheat starch which has been ground in a ball mill until the granules are completely ruptured). Since much of this fraction is apparently derived from the available starch by the action of beta-amylase, it is tentatively designated as amylopectin.

Tube No. 1 (Fig. 1) contains the amylopectin and starch centrifuged from 50 g. of an Oregon soft-wheat flour (protein 7.5%); No. 2 from experimentally milled 85% patent flour (protein 12.5%); No. 3 from 85% baker's patent flour (protein 12.5%); No. 4 from clear flour (protein 14.1%); and No. 5 from durum flour (protein

13.8%). The flour represented by No. 2 was experimentally milled from a sample of the same wheat mix from which the flour represented by tube No. 3 was milled. It will be noticed that the ratio of amylo-dextrin to starch increased with the hardness of the wheat from which the flour was milled, that is, from soft wheat to hard wheat to durum. This increase in amylo-dextrin with increase in hardness may be an inherent characteristic of the starches as they occur in the wheats, or it may indicate that the ratio of amylo-dextrin to starch increases with the severity of the milling. This latter hypothesis is substantiated by the smaller amount of amylo-dextrin obtained from the experimentally milled flour than from the commercially milled flour. Further substantiation is given in Figure 2, which shows the starch and amylo-dextrin obtained from a baker's patent flour (tube A) in comparison with that washed from a sample of the middlings from which the flour was ground (tube B). There was much less of the amylo-dextrin fraction from the latter than from the former.

After decanting the liquid from the centrifuge tubes, the starch and amylo-dextrin may be remixed and dried together or they may be separated and dried individually at room temperature under a fan.

### Baking Results with Synthetic Doughs

If the starch, amylo-dextrin, and wet gluten from a flour are recombined in a dough, baking characteristics similar to those of the original flour are obtained. Figure 3 shows loaves baked from two hard-wheat, unbleached, experimentally milled flours as contrasted

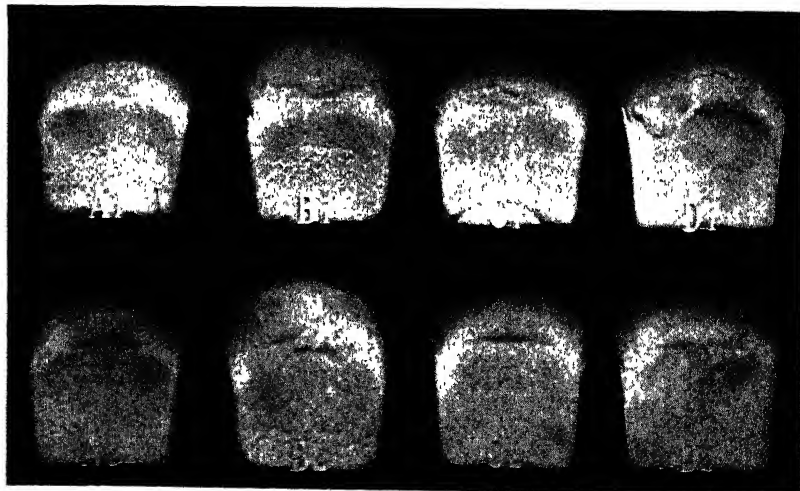


Fig. 3. Comparison of loaves baked from flour with loaves baked from recombined gluten, starch, and amylo-dextrin.

with those baked from the recombined starch, amyloextrin, and wet glutens. Loaves  $A_1$  and  $B_1$  represent one flour: basic (volume 550 cc.) and plus 1 mg.  $KBrO_3$  (volume 605 cc.) respectively. Loaves  $A_2$  (volume 550 cc.) and  $B_2$  (volume 635 cc.) are the corresponding synthetic loaves. Loaves  $C_1$  and  $D_1$  were baked from a flour with different characteristics,  $C_1$  being the basic loaf (volume 515 cc.) and  $D_1$  the oxidized loaf (volume 570 cc.).  $C_2$  (volume 530 cc.) and  $D_2$  (volume 560 cc.) are the comparable loaves baked from the starch, amyloextrin, and wet gluten.

These comparative results show that there is a similarity between the "synthetic" loaves and the loaves baked from the original flours and permit the conclusion that the results obtained by this method reliably reflect the properties of the flour constituents as they occur in the original flour. This would also indicate that those water-soluble constituents removed in washing the gluten and starch are either not essential or that they exert their effect before being washed out.

### Baking Procedure

If wet crude gluten is washed and squeezed free from excess water by one operator, it is found to be quite consistent in composition from day to day and even from one flour to another. As prepared in this laboratory the gluten usually contains 68% to 70% water and about 27% protein. The starch as dried contains about 10% moisture. In the following studies on the properties of the starches, amyloextrins, and glutens an arbitrary formula was used to provide dough which approximated that from an 11.5% gluten flour. The formula is as follows: 42 g. of wet crude gluten containing 29 cc. of water and 11.3 g. of protein is combined with 77 g. of starch (dry weight) or the same quantity of starch plus amyloextrin. The absorption of this dough when starch plus amyloextrin is used is about 60% to 65% on a 15% moisture basis, practically the same as that of a corresponding flour. Seven grams of sugar in these synthetic doughs has been found amply sufficient for a three-hour fermentation.

The technique of mixing the wet gluten with the starch and other ingredients is as follows: The ingredients including about two-thirds of the starch are placed in the Hobart-Swanson mixer. The gluten is soon disintegrated and the mixture becomes exceedingly slack; then the remaining starch is added. It is necessary to loosen the starch that becomes caked on the bottom of the mixer bowl and to strip the dough from the mixer pins whenever it begins to "ride." The mixing is continued until the bowl cleans and the dough becomes smooth. The total time spent in the mixing operation is usually four or five minutes.

If dry gluten is to be used, it must be dried at room temperature as rapidly as possible (Aitken and Geddes, 1938). Hanging the wet gluten in a thin sheet over a cord stretched between supports offers a maximum of drying surface to the breeze from a fan (DuBois, Hutton, and Moxon, 1938). The dried gluten is ground in a burr or coffee-type mill.

Since coarsely ground dry gluten hydrates slowly and consequently must soak sometime before the final mix, a sponge method or a soaker method is used. In the sponge-dough method, the ground gluten is mixed with the starch; then the other ingredients are added and mixed to a paste with a spatula; this is given  $1\frac{1}{2}$  hours' fermentation to allow for the hydration of the gluten and then remixed in the Swanson mixer to a smooth dough. It is then given another hour and a half fermentation with a punch at one hour.

In the soaker procedure the gluten alone is mixed with the water and allowed to soak for two hours. By this time the gluten has assumed the character of ordinary wet gluten and consequently the mixing procedure is the same as that for wet gluten and starch.

### Water Absorption

It was shown in Figure 3 that if the gluten, starch, and amylo-dextrin are recombined in a dough, the baking characteristics are similar to those of the original flour. If the amylo-dextrin fraction is left out of the dough, the characteristics are considerably changed. The absorption drops about 10%. This suggests that the available starch, the precursor of the amylo-dextrin fraction of the starch, is a factor influencing the absorption of flour. Alsberg (1935) postulated that this might be the case. (See also Pulkki, 1938.) As is well known, differences in protein content are also factors affecting the absorption. As was shown in Figure 1, soft-wheat flours yield considerably less of the amylo-dextrin fraction than the hard-wheat flours, and the durum flours yield much more; this corresponds with the well-known differences in the absorptions of these flours. Durum starch (minus the amylo-dextrin) gives an absorption about 7% above that obtained when the other starches are used. But with this exception, the differences found in the absorption of the glutes or of the undamaged starches (excluding the amylo-dextrin fraction) of the different flours which we have used have been insignificant, accounting for less than 1% of variations between flours. Shollenberger and Coleman (1926) working with wheat middlings ground to varying degrees of fineness showed that water absorption had a very marked tendency to increase with each increase in degree of fineness of grinding and that this tendency was very much more pronounced with the

hard than with the soft wheats. This is further evidence that the amount of damage to the starch in milling is dependent on the hardness of the wheat. It is noteworthy (Fig. 4) in this connection that purified middlings (from a hard-wheat mix from a commercial mill) that were fine enough to pass a 50GG bolting cloth and coarse enough to be retained on a 70GG cloth were baked (with  $\text{KBrO}_3$ ) into an excellent loaf of bread but the absorption was low—in this case only 55%—while the absorption of the flour milled from a sample of these middlings had an absorption of 60%. A comparison of the starches and amylo-dextrins from these middlings and from the flour was shown in Figure 2. This confirms the conclusion that the amyloextrin fraction of a flour is greatly affected by the severity and fineness of grinding and that the amyloextrin fraction in its turn affects the absorption.

Figure 4 shows the comparative characteristics of the loaves baked from the flour and from the middlings. Loaves *A* and *B* represent the flour, basic and with 1 mg.  $\text{KBrO}_3$  respectively. Loaves *C* and *D* are from middlings correspondingly treated.

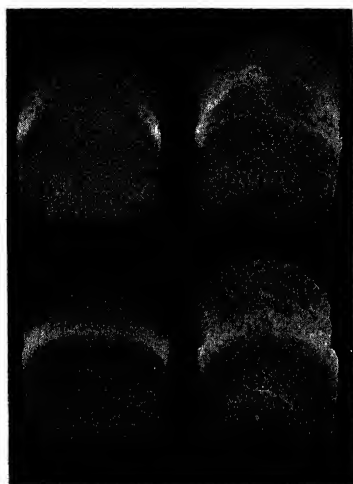


Fig. 4. Comparison of loaves baked from flour and from middlings.



Fig. 5. Effect of the amyloextrin on loaf characteristics.

### Handling Characteristics of Synthetic Doughs

The handling properties or "feel" of a dough made from starch, amyloextrin, and gluten are similar to those of a dough made from flour. But if the amyloextrin fraction is left out, the handling properties are completely changed. The amyloextrin-free doughs are not sticky even when they are exceedingly slack. Stickiness in doughs seems to be associated with this amyloextrin fraction. Dur-

ing fermentation there is always a surface layer of moisture on the amyloextrin-free dough, as though some syneresis were occurring. The absorption is also quite critical, small differences in the water added making a big difference in slackness. These amyloextrin-free doughs bake into loaves with greater oven spring and greater volume than the doughs containing the amyloextrin. The loaves shown in Figure 5 illustrate the characteristic effect of the amyloextrin fraction on loaf characteristics. Loaf *A* was baked from gluten plus starch without the amyloextrin fraction while loaf *B* was baked with starch plus the amyloextrin. The drastic effect produced by the addition of some samples of amyloextrin is illustrated in Figure 6. Loaf *A*

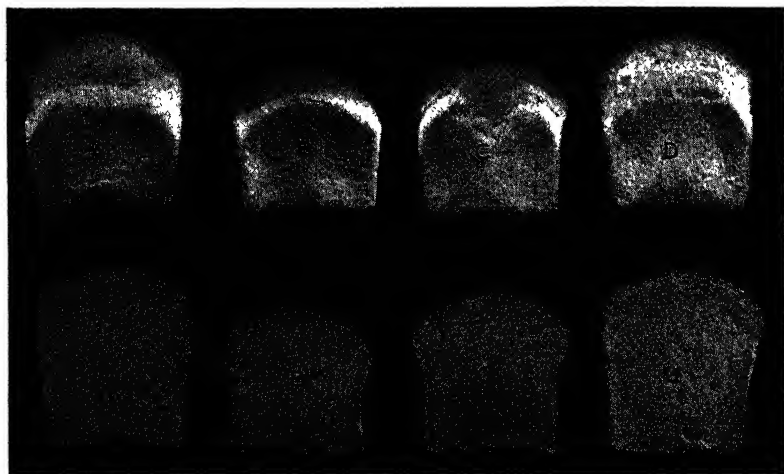


Fig. 6. Effect of amyloextrin on loaf characteristics.

was baked from gluten and starch without amyloextrin, loaf *B* contained the amyloextrin, loaves *C* and *D* contained the amyloextrin but with the addition of 1 mg. of  $\text{KBrO}_3$  in *C*, and the water extract of one gram of malted wheat flour in *D*. From the appearance of the loaves one might suspect that the amyloextrin acted as a reducing agent but this does not seem to be the case since oxidation does not eliminate the effect.

The presence of the amyloextrin fraction of starch in synthetic doughs has a very marked tenderizing effect on the crumb of the baked loaf. The crumb of those loaves baked from gluten and starch without the amyloextrin is decidedly tough and "rubbery" while the crumb of loaves containing the amyloextrin is similar in tenderness to loaves baked from flour.



### Effect of Malt

Malt is used in baking not only to increase the gassing power of the flour but also because of its peculiar ability under some conditions to improve the gas-retaining properties of flours. This improvement has long been attributed to a softening effect on the gluten or, in other words, to its proteolytic action. It is noteworthy in this regard that some unbleached flours which supposedly, according to the theory of Balls and Hale (1936) and Jørgensen (1935), would require oxidation to inhibit proteolysis are remarkably improved by malt treatment. In fact it is quite difficult to distinguish the improving effect of malt from oxidation on some flours. Figure 7 shows pictures of loaves of bread baked from an unbleached 85% baker's patent flour with a

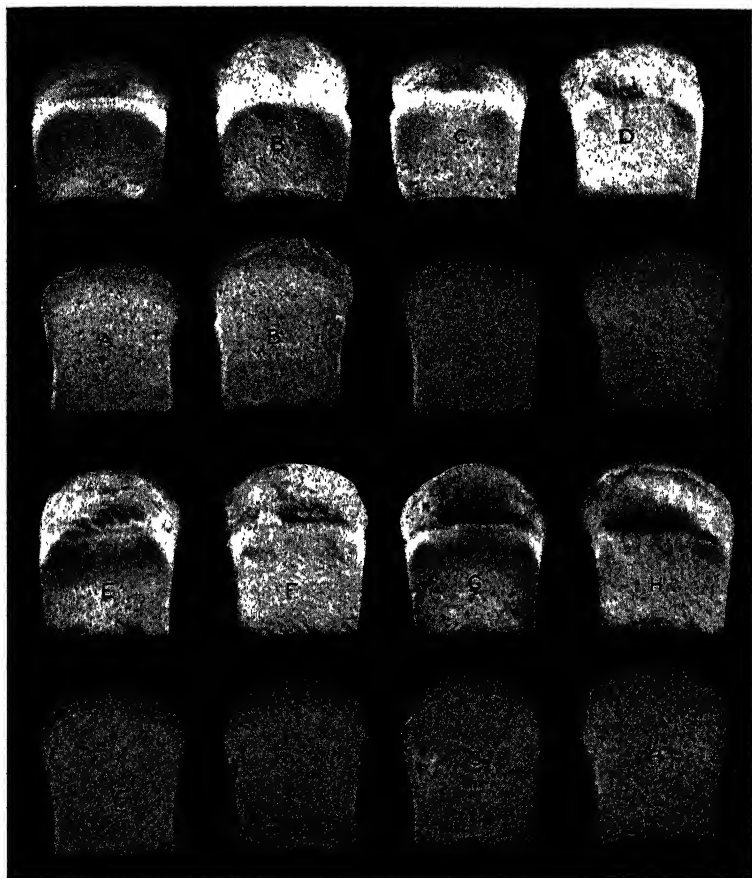


Fig. 7. Comparison of bromate effect and malt alpha-amylase effect on an unbleached baker's patent flour.

protein content of 12.5% with increasing increments of malt alpha-amylase.

Loaf *A* (volume 530 cc.) is the basic loaf with no alpha-amylase, loaf *B* (volume 630 cc.) had 1 mg. of  $\text{KBrO}_3$ ; *C* (volume 570 cc.) had alpha-amylase equivalent to 0.5% of malt; *D* (volume 635) had 0.5% malt equivalent of alpha-amylase plus 1 mg. of  $\text{KBrO}_3$ ; *E* (volume 650) had 2½% malt equivalent of alpha-amylase; *F* (volume 715) had 2½% malt equivalent alpha-amylase plus 1 mg.  $\text{KBrO}_3$ ; *G* (volume 675) had 6% malt equivalent of alpha-amylase; and *H* (volume 750) had 6% equivalent plus 1 mg.  $\text{KBrO}_3$ .

It is seen that this flour, even though needing and responding to oxidation, was stimulated by malt alpha-amylase. There was an excess of sugar in all of these bakes—5% sucrose added to the flour

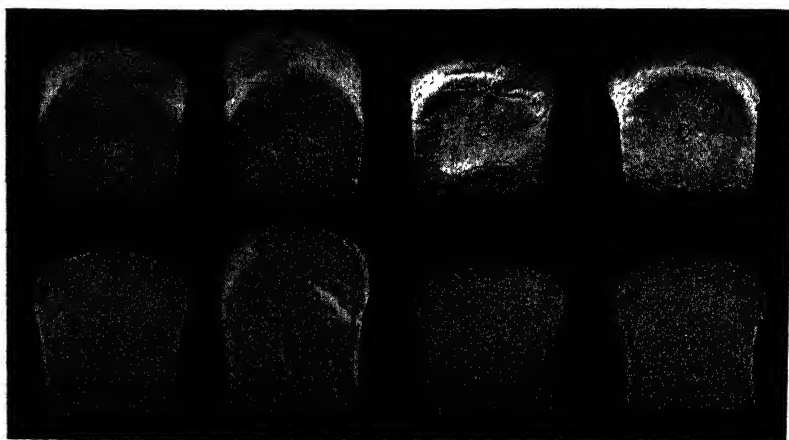


Fig. 8. Differences in starch response to malt alpha-amylase treatment.

with a maltose value of 190. The alpha-amylase-treated, unoxidized loaves *E* and *G* have an appearance and volume similar to the oxidized loaf (*B*). The effect appears to be the result of the action of alpha-amylase on the starch and not the result of proteolysis.

All flours do not respond equally to the addition of malt or malt alpha-amylase. If the effect is on the starch it would be expected that the starches from these flours would give a corresponding response in synthetic doughs. That starches respond differently to malt extract is shown in Figure 8. These loaves, baked from starch-gluten doughs, all contain 42-g. increments of the same wet gluten. Loaves *A* and *B* contain 86 g. each of one sample of starch, without and with the addition of the water extract from 1 g. malted wheat flour. Loaves *C* and *D* were similarly treated but contained a starch which did not respond to malt treatment. This same type of result is obtained

regardless of whether malt flour, malt extract, or malt alpha-amylase is used. These results indicate that the starch and not the gluten characteristics determine the amount of stimulation by malt alpha-amylase.

Certain flours contain starch which responds more readily than others to malt treatment. The Chiefkan flours which we have investigated are conspicuous in this respect. Figure 9 shows loaves baked from Chiefkan flour (*A*, *B*, *C*, and *D*) and from Chiefkan starch and gluten (*E*, *F*, *G*, and *H*); *A* and *E* are the basic loaves, while *B* and *F* contained malt extract equivalent to 1% of malt. *C* and *D* are the bromated-flour loaves, without and with, respectively, the equivalent of 1% malt. *G* and *H* are the synthetic loaves, correspond-

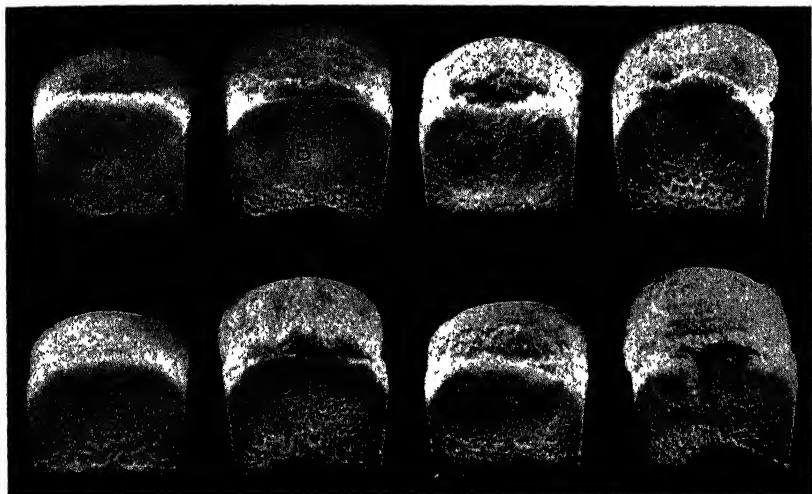


Fig. 9. Response of Chiefkan flour and Chiefkan starch to malt extract.

ing to *C* and *D*. It will be noticed that this Chiefkan flour was stimulated by malt but did not give a good response to oxidation.

That some of the undesirable characteristics of flours of certain exceedingly hard wheats, such as durum and possibly Chiefkan, may be attributed to the starch and probably to the damage done to the starch in the milling process is shown in Figure 10. These loaves all contain 42-g. increments of the same baker's patent flour gluten. The starch used in loaves *A*, *B*, *C*, and *D* was washed from durum flour while that used in loaves *E*, *F*, *G*, and *H* was washed from durum semolina. Loaves *A*, *B*, *E*, and *F* are the amyloextrin-free loaves; while *C*, *D*, *G*, and *H* contain the amyloextrin. Loaves *B*, *D*, *F*, and *H* were treated with 1% malted wheat flour. The differences between loaf *A*, from durum-flour starch, and loaf *E*, from durum-

semolina starch, would seem to be due to the damage done to the starch in milling the flour from the semolina. The loaves shown in Figure 4 indicate a similar though less pronounced tendency between the middlings and flour from a commercial hard-wheat mix. Comparing loaves *B* and *F* (Figure 10) it is seen that the malt treatment virtually eliminated the differences. In comparing loaves *D* and *H* with *B* and *F* it is seen that though a malt treatment overcomes some of the effect of the amyloextrin, the loaves are still not as good as the malt-treated, amyloextrin-free loaves.

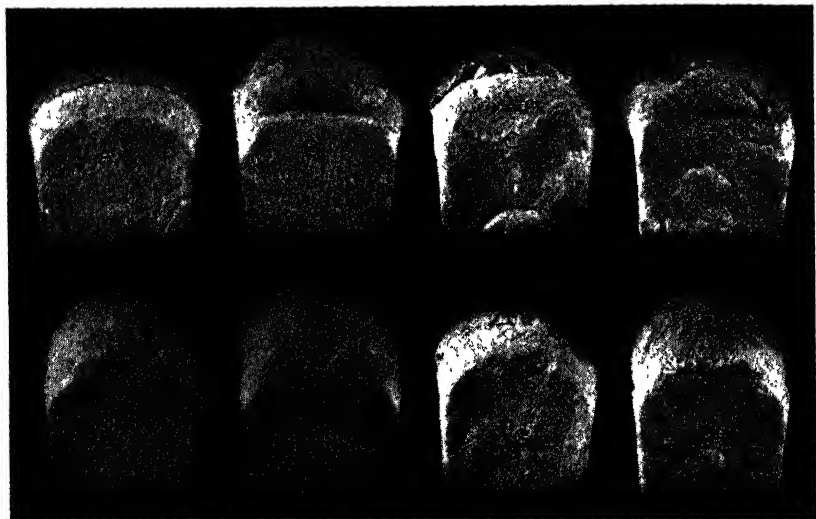


Fig. 10. Comparison of loaves baked from durum flour starch and durum semolina starch.

In connection with the improving effect of malt on the starches, its effect on the "commercial" wheat starch, which we found had exceedingly poor baking properties, should be noted. Figure 11 shows loaves baked from doughs using a baker's patent flour gluten in combination with "commercial" wheat starch. The starches used in this series, in the order in which they occur in the figure, were given a 4-hour preliminary treatment with malt alpha-amylase equivalent to 0%, 1%, 2%, and 4% of malt respectively. The treated starches were then redried and used for these bakes. The volumes of the loaves increased from 415 cc. to 690 cc. That the alpha-amylase in this case must be given considerable time in which to act is shown in Figure 12. The commercial starch used in loaf *A* had no preliminary treatment but an alpha-amylase solution equivalent to 4% malt was added to the dough at the mix.

The starch used in loaf *B* (Fig. 12) had the four-hour preliminary treatment with the same quantity of alpha-amylase. These results further indicate that the improving action of malt is on the starch and not on the gluten since the malt alpha-amylase was acting for the same length of time on the gluten in these two loaves. Since commercial wheat starch is given a treatment with hypochlorite in the process of purification it was thought that this might possibly be the treatment which caused its detrimental properties when it is used in dough. Accordingly laboratory-prepared starch was suspended in water and chlorine gas added. This chlorine treated starch when dried had similar properties to the commercial-starch and it also was improved by malt treatment. No explanation of this improvement by malt is apparent.

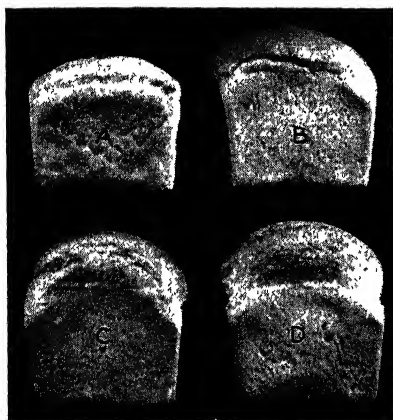


Fig. 11. Loaves baked from a commercial wheat starch with increasing malt alpha-amylase treatment.

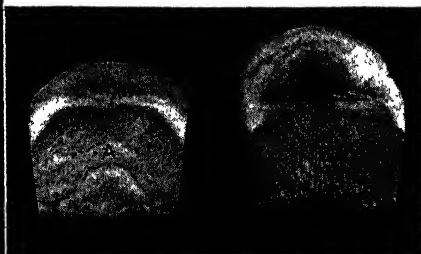


Fig. 12. Effect of time on response to malt alpha-amylase.

### Summary

Starch and gluten which have been washed from flour may be recombined to form a dough with baking characteristics similar to those of original flour doughs. Various combinations of starch and gluten fractions, and of various treatments, can be suitably studied by this technique.

The starch from flour may be separated into two fractions by centrifuging. One of the fractions is relatively pure starch and the other (smaller fraction) contains the dextrins produced by the action of the amylases of the flour on the readily available starch; this fraction is tentatively designated as amyloextrin.

The amyloextrin fraction of the flour is a factor in absorption and determines to a marked extent the handling characteristics of

flour doughs. It is closely associated with stickiness of doughs and with tenderness of crumb in the baked loaf.

The improving effect of malt on many flour doughs is due not to its proteolytic action but to the action of alpha-amylase on the starch.

Certain undesirable baking characteristics of some exceedingly hard wheat starches are due to damage to the starch in milling.

Some commercial wheat starches seemingly owe their poor baking properties to hypochlorite treatment. The detrimental effect of the hypochlorite may be overcome by treatment with large quantities of malt, the effective agent apparently being the alpha-amylase component of malt.

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## A COMPARISON OF METHODS FOR THE DETERMINATION OF PROTEOLYTIC ACTIVITY<sup>1</sup>

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(Read at the Annual Meeting, May 1939)

Investigations of the relative utility of various methods for determining the proteolytic activity of cereal products have been reported from time to time by Cairns and Bailey (1928), Brownlee and Bailey (1930), Herd (1931), Tissue and Bailey (1931), and Laufer (1937). Of the various procedures studied, the copper-precipitation method of

<sup>1</sup> Paper No. 15, Journal Series, General Mills Research Laboratories. Sub-committee report, 1938-39, Committee on Methods of Analysis.

Ritthausen (1872) as modified by Blish (1918), and later by Olsen and Bailey (1925), and the Sørensen formol titration appear to be the most suitable.

Recently two new methods have been proposed, the gelation-rate procedure of Landis and Frey (1938) and the viscometric method of Koch, Nelson, and Ehrnst (1939). The present study was designed to compare the results obtained with these procedures with those given by the copper-precipitation technique and the formol titration as modified by Samuel (1934). The experiments were conducted in two series. Initially, the Ritthausen method and the modified Sørensen procedure were compared.

Since it was found that both methods gave essentially the same results, the Sørensen technique alone was used in the second series in comparison with the gelation-rate and viscometric methods. To afford information as to the effect of variation in substrate, in the second series the formol titration was used with both flour and gelatin.

### Experimental

For the comparison of the Ritthausen copper-precipitation and formol titration methods six samples of southwestern patent flours from the 1937 crop year were tested. In the second comparison fifteen samples covering a reasonably wide range of cereal products were studied by the four methods mentioned above. A description of the second series of samples is included with the data in Table II.

The Ritthausen method was used essentially as described by Blish (1918) and Olsen and Bailey (1925). Autolytic digestions were carried out at 40°C. for 48 hours.

For comparison with the copper-precipitation method, the same samples were also digested for 48 hours at 40° and the increase in amino nitrogen determined by means of the Sørensen formol titration as modified by Samuel (1934).

In preliminary experiments with the rate-of-gelation procedure as described by Landis and Frey (1938), difficulty was encountered in preparing replicate gelatin dispersions of reasonably uniform gelation characteristics. This trouble was overcome by resorting to a technique suggested by the work of Davis, Oakes, and Browne (1921). The desired amount of gelatin was placed in a tared flask, the proper amount of buffer and water added, and the mixture placed in an ice box at 5°C. for 7 to 8 hours. At the end of this time the gelatin was dispersed by heating the mixture to exactly 75°C. in a water bath held constant at 80°C. After cooling to approximately 40°C., sufficient water was added to bring the total to the desired weight and the dispersion then placed in the 30° constant-temperature bath. In other

respects the procedure as used was precisely that described by Landis and Frey (1938).

The viscometric procedure reported by Koch, Nelson and Ehrnst (1939) was also somewhat modified. It was found that a 7.5% concentration of gelatin gave a more satisfactory dispersion with the particular gelatin employed. In the calculation of results the unit of proteolytic activity was taken as 100 times the reciprocal of the time necessary to reach a 20% drop in viscosity. This form of calculation seems preferable since it gives rise to values which are directly proportional to the concentration of the material used and hence presumably to the enzyme concentration. It was subsequently found that this

TABLE I  
COMPARISON OF RITTHAUSEN AND FORMOL TITRATION METHODS

Sample No.	Averages of triplicate determinations	
	Ritthausen method, N not precipitated by $\text{Cu}(\text{OH})_2$	Formol titration— increase in amino N
	<i>Mg. per 10 g.</i>	<i>Mg. per 10 g.</i>
	A	B
1	3.82	0.25
2	4.80	0.49
3	4.36	0.42
4	6.46	0.60
5	8.02	0.74
6	3.00	0.35
$r_{AB} = +.990$ 5% pt. = 0.754 1% pt. = 0.874		

TABLE II  
COMPARISON OF PROTEOLYTIC ACTIVITY VALUES

No.	Sample Description	Landis and Frey		Koch, Nelson, and Ehrnst		Formol titration—increase in amino N			
						Gelatin		Flour	
		<i>Milliunits per g.</i>		<i>Units per g.</i>		<i>Mg. per 10 g.</i>		<i>Mg. per 10 g.</i>	
1	Patent, A mix	0.3	0.3	1.2	1.1	1.2	1.3	0.4	0.4
2	1st clear, A mix	0.8	1.0	2.0	1.8	2.0	2.0	0.6	0.9
3	2nd clear, A mix	3.8	3.5	2.3	2.1	3.0	3.4	1.3	1.3
4	Patent, B mix	0.5	0.6	1.9	1.9	1.3	1.3	0.4	0.6
5	1st clear, B mix	1.3	1.2	3.4	3.2	2.7	2.3	0.8	0.9
6	2nd clear, B mix	5.0	4.7	4.3	4.0	3.0	3.0	1.4	1.3
7	Bran, A mix	3.9	3.5	3.6	4.0	3.0	4.2	5.0	5.0
8	Red Dog, A mix	7.0	9.2	7.1	6.5	6.6	5.3	2.3	2.2
9	Malt flour 1	2.1	2.0	1.8	1.9	2.0	2.0	1.5	1.0
10	Ground whole malt	3.9	3.9	3.0	2.9	5.4	5.1	3.4	2.9
11	Malt flour 2	6.5	5.9	4.2	3.8	3.7	3.6	2.9	2.5
12	Ground acrospires and roots from malt	10.4	10.2	6.3	5.9	32.8	36.8	16.8	12.9
13	Wheat germ 1	14.3	15.6	5.6	5.3	3.1	3.6	4.4	3.8
14	Wheat germ 2	10.8	12.1	5.6	4.8	4.4	4.3	3.6	4.1
15	Papain	$3.3 \times 10^4$	$3.6 \times 10^4$	$2.5 \times 10^3$	$2.3 \times 10^3$	$2.8 \times 10^3$	$2.8 \times 10^3$	$8.8 \times 10^3$	$9.0 \times 10^3$



form of expression gave results consistently in better agreement with those of the other methods.

The formol titration procedure used with the second series of samples in comparison with the rate-of-gelation and viscosity methods was essentially that described by Samuel (1934). In one instance a 3% gelatin dispersion in *M*/20 acetate buffer of pH 5.0 was used as substrate. In the second case, the substrate was a 10% suspension of a southwestern patent flour in the same buffer. To both substrates were added aqueous extracts, usually 20%, of the material to be tested. Duplicate digestions were carried out at 40°C. for 48 hours, together with appropriate control determinations in each case for both the substrate alone and for the extract alone.

The results obtained are shown in Tables I and II and an analysis of the data obtained for the second series of samples is given in Tables III and IV.

TABLE III  
CORRELATION COEFFICIENTS FROM DATA OF TABLE II

	Landis and Frey	Koch, Nelson, and Ehrnst	Formol gelatin
Koch, Nelson, and Ehrnst	+ .855	—	—
Formol gelatin	+ .413	+ .512	—
Formol flour	+ .554	+ .590	+ .947
5% pt. = 0.532			

TABLE IV  
SUMMARY AND ANALYSIS OF DATA FROM TABLE II

	Landis and Frey	Koch, Nelson, and Ehrnst	Formol titration— increase in amino N	
			Gelatin	Flour
	<i>Milliunits per g.</i>	<i>Units per g.</i>	<i>Mg. per 10 g.</i>	
Mean	5.58	3.92	5.88	3.28
Range	0.3–15.6	1.1–7.1	1.2–36.8	0.4–16.8
Standard error of single determination	0.16	0.16	0.21	0.27
Coefficient of variation, %	2.87	4.08	3.57	8.23
ANALYSIS OF VARIANCE				
Variance between duplicates	0.02577	0.02638	0.04400	0.07462
Variance between samples	38.241	5.7949	136.73	25.989
F	1484.0	219.67	3107.5	348.28
5% pt.	2.55	2.55	2.55	2.55

### Discussion

The data given in Table I<sup>2</sup> show that the Ritthausen method and the formol titration procedure as modified by Samuel give essentially

<sup>2</sup> I am indebted to Mr. H. F. Vaupel for making these determinations and for his assistance in preliminary studies with the Landis and Frey method.

similar results. This is in agreement with the conclusions previously reached by Tissue and Bailey (1931) and by Blish (1918).

The analysis shown in Tables III and IV was computed from results with only the first 14 samples for all methods. The last sample, which was one of papain, was so far removed from the range of activity of the other materials tested as to influence unduly any analysis of data in which it was included.

The correlation coefficients indicate that the gelation-rate and viscometric methods give results which are in good agreement between themselves. The same is true of the formol titration procedures using gelatin and a flour suspension as substrates. In contrast the correlation is relatively poor between either the gelation or viscometric procedures and either type of formol titration. This suggests that the physical methods (gelation-rate and viscometric) are measuring a different type of activity from that tested by either the formol titration or copper-precipitation procedures. It may reasonably be assumed that the physical methods measure proteinase activity—that is, degradative or disaggregative action on the whole protein—whereas the other methods are based on an increase in products of low molecular weight, which would be produced to a greater extent by the action of dipeptidases and/or polypeptidases. It has always been assumed that proteinase activity is of the more importance in relation to dough fermentation problems, and in such connection the gelation-rate or the viscometric method would be the more suitable.

In referring again to the original data, it seemed possible that much of the difference in results between these two classes of methods might be ascribed to the values given by sample No. 12. Correlation coefficients were again calculated omitting this sample and all were found to lie within the range  $+ .82 \pm .05$  except that between the gelation-rate and formol-gelatin procedures which was  $+ .58$ . Clearly, the distribution of the protease systems in samples other than No. 12 is essentially the same. It should not be inferred, however, that these types of methods may be used interchangeably, since a system similar to that of sample No. 12 may be encountered in other materials.

The relative precision of the several procedures may be seen from the values given in Table IV for the standard error of a single determination. These values are not directly comparable since the units by which activity is expressed in the several methods are not the same. In order to facilitate comparison the coefficient of variation—the standard error as percent of the mean for all samples—is also given. Another form of expression of the experimental error is the variance between duplicates shown under the analysis of variance.

The relative ability of a method to differentiate between samples is governed by both the experimental error and the variability between means of samples; the larger the variance between sample means and the smaller the experimental error the greater will be the distinction between samples. A measure of this differentiation is given by the *F* values, which are an expression of the ratio of the variance between samples to the variance between duplicates. While these values are not directly comparable they clearly indicate that as between the viscometric and rate-of-gelation procedures the latter is to be preferred since it gives a much greater distinction between samples. By the same reasoning the use of gelatin as a substrate in the formol titration method is to be preferred to flour since the experimental error is lower and the differentiation between samples higher.

Of the procedures studied, the formol titration method is least time consuming, while there is little to choose in this respect between the gelation-rate, viscometric, and copper-precipitation methods. The gelation-rate procedure has the serious disadvantage of requiring a considerable amount of highly specialized and expensive apparatus, which is not the case with the other methods.

### Summary

The rate-of-gelation and viscometric methods of determining proteolytic activity recently proposed by Landis and Frey (1938) and by Koch, Nelson, and Ehrnst (1939) have been investigated in comparison with the copper-precipitation and formol titration procedures, using in the last case both gelatin and a flour suspension as substrates. This comparison has been carried out with a series of samples comprising a reasonably wide variety of cereal products.

With the limited number of samples tested, the gelation-rate and viscometric methods gave results substantially different from those obtained with the other procedures. It may be presumed that the first two methods measure proteinase activity, while in the other types of procedure dipeptidase and/or polypeptidase action have the most effect on the results obtained.

In these experiments, the gelation-rate and viscometric methods had the same experimental error, but the former seems preferable because of its better differentiation between samples. The use of the gelation-rate procedure is limited by its requirement of relatively elaborate and costly apparatus.

The modified Ritthausen copper-precipitation and formol titration methods used were found to give essentially the same relative results.

In the formol titration procedure the use of gelatin as a substrate seems preferable to that of flour since this technique leads to lower experimental error and better differentiation between samples.

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## CHANGES IN FLOUR ON STORAGE WITH SPECIAL REFERENCE TO THE EFFECT OF DIFFERENT TYPES OF BAGS

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(Read at the Annual Meeting, May 1939)

Flour storage in different types of containers has been the subject of several researches. Swanson, Willard, and Fitz (1915) found that storage in sealed jars or in cloth sacks made little difference in its behavior as to baking quality during storage. Miede (1933) studied the maturing of wheat and flour in bags, in bulk covered at 21°C., in air day and night, and in hermetically sealed bottles. He concluded that the tenacity and baking value of the milled flour was greatly improved by storage and that the tenacity of the gluten was greatest in the closed samples after six months of storage and also that the amount of moisture was greater in samples left in the open air in bulk.

Kuhl and Kliefoth (1934) found that the keeping quality of a flour stored for six months was better when it was compressed into a solid mass than when stored in the usual form in cloth sacks.

Krtinsky (1937) studied flour storage in jute and paper bags. The factors considered were moisture, gluten content, and gluten quality over a period of three months. Krtinsky concluded that at room temperature the changes incident to storage were similar for flour stored in these two types of containers, both of which were found satisfactory. However, he found a greater rate of moisture loss in jute bags than in paper bags, while gluten content, gluten quality, and increase in absorption were the same during the storage period in the two bags.

Thiessen (1933) found that the absorption of Wyoming hard-wheat flours increased 6% to 10% during 12 months of storage in cotton sacks, and only 0% to 2% during 21 months storage in cans.

Fisher, Halton, and Carter (1937) stored flours in cubical sealed tins and bags and studied the effect of storage over a period of 18 months. They found that "the bagged and tinned dry (12% moisture) samples showed closely similar changes in chemical composition during 18 months' storage, and the changes were by no means marked. On the whole the tinned dry samples showed smaller changes than the corresponding bagged (and slightly moister) samples." The fact that the bagged samples were slightly more moist may account for this. The gluters from the bagged and tinned dry samples of English patent showed very little change either in quality or quantity.

Although the intention in the present research was to study the effect of cotton, jute, paper-lined, and grain bags on the storage of flour, certain factors of significance have been found during the course of the tests which have extended the scope of the findings to include observations of the general effect of storage of flour on absorption, ash, protein, flour weight, and baking quality. In view of this, it is probably advisable to review some of the previous researches on these factors. The following citations are somewhat confusing, thus indicating the need for further research.

Bailey (1925) has summarized the literature up to 1925 in regard to the changes in flour incidental to aging. The early researches in regard to the effect of humidity on the weight of flour, summarized by Bailey, point out first that because of the fine state of division and the greater exposure resulting from the method of handling, flour responds more readily to changes in humidity of the storage room than does bulk grain; second, these researches demonstrate the hygroscopicity of flour; and third, they show that the amount of moisture in flour varies with the humidity of the storage room in which it is stored. Arpin and Pecaud (1923) found that small packages of flour showed greater change in weight than did larger packages when stored in the same atmosphere.

Bailey (1925) mentions that various investigators have shown that natural aging of flour results in an improvement of the color score due

to the "spontaneous oxidation of the carotinoid pigments." Swanson, Willard, and Fitz (1915) found that freshly milled flour reached its maximum color score in 305 days whether stored in heated or unheated rooms and then in some samples fell off slightly up to 366 days. However, Saunders, Nichols, and Cowan (1921) found that wheat stored and baked at various intervals reached a maximum color score in three years and maintained about the same level for ten years.

Sharp (1924) has shown the effect of moisture content and temperature on the keeping qualities of flour as indicated by changes in acidity. His work shows that lower temperatures and lower moisture contents favor longer keeping qualities, while higher temperatures and higher moisture contents show that flour will remain sound for much shorter lengths of time. Also, flour with a moisture content of 14.4% would remain sound only if kept at abnormally low temperatures. However, Reimund (1916), as reported by Bailey, believed that flour could be stored at 18°C. without spoiling if the moisture content did not exceed 15%. Mangels (1924) gives evidence supporting the belief that warm storage of flour is more detrimental than cool storage. The volumes of loaves from flour stored in warm storage showed a greater decrease after three or four months than did the flour from cool storage. Halton and Fisher (1937) have found that flour can be safely stored and exported even into tropical countries if the moisture content is not above 12% and that it is not necessary to go below 12% in order to insure safe storage. Greaves and Hirst (1925) state that highly milled flour from sound wheat can be stored in dry rooms free from odors for at least four years without deteriorating; however, poor-grade flour deteriorates more rapidly.

Many writers have investigated and emphasized the tendency of bacteria, mold, and insects to grow in flour in the higher-moisture brackets at warm temperatures, and of course almost every baker realizes the danger of storing moist flour at warm temperatures.

Several investigators have conducted researches into the effect of storage of flour on baking quality. All of the investigations show an improvement in coloring and strength and general baking qualities on storage. The most extensive work has been reported by Swanson, Willard, and Fitz (1915) and Saunders, Nichols, and Cowan (1921). Swanson, Willard, and Fitz studied the effects of storage on the baking quality of flour stored in sealed jars and in cloth sacks. The baking quality (loaf volume and "gluten quality factor") increased up to about 125 days or more and then diminished up to 366 days. However, the baking tests indicated that the "strength" at the beginning of the test was not so good as after 366 days. Bleached flour was included in the series and showed about the same trend as the unbleached samples.

Saunders, Nichols, and Cowan found that flour showed an increase in loaf volume and baking strength for two to three years. This was then followed by a decrease. Yet at the end of twelve years' storage the data show baking strength superior to that at the start of the experiment. Their data indicate that flour stored under good conditions can be kept for ten years.

The experiments of Saunders, Nichols, and Cowan (1921) show an increase in absorption with time of storage; at the end of about thirteen years the increase in absorption was enormous (about a 24% to 25% increase). Stockham (1917) reported that fresh flour increased in absorption 1.2% after eight months storage.

Greaves (1925) confirms the fact that water-absorption power of flour is increased during storage. He also reports that some flours yielded a loaf of greater volume after storage whereas others registered a smaller volume. He concludes that on the whole bread-making flour is improved in baking quality on storage.

Thiessen (1933) studied the effect of aging on Wyoming hard-wheat flours stored in both cotton sacks and tightly closed cans and concluded that an aging period of from one to three months improved flour and after three months there was little further change for about two years, but after two years deterioration set in and increased rapidly to the four-year period. Throughout the storage period the water-absorption power of the flour increased and was most rapid when the flour was stored in sacks.

Kuhl and Kliefoth (1934) found that flour treated with  $\text{KBrO}_3$  and electrically bleached does not keep as well as untreated and unbleached flour and that the electrically bleached and treated flour falls off somewhat in baking quality on storage.

Kozmin (1935) stored flour at various temperatures in hermetically sealed jars. After three months, flour stored at  $15^\circ\text{C}$ . showed practically no change in the quality of the gluten, while flour stored at  $30^\circ\text{C}$ . and  $45^\circ\text{C}$ . showed the effect of aging. Kozmin found that aging strengthened the gluten and that this took place much more rapidly at the higher temperatures. The flour was not chemically aged or bleached.

Kent-Jones (1924) reports the changes in flour or aging for five months as follows: (1) the ash showed little or no change; (2) nitrogen scarcely changed; (3) the crude gluten tended to decrease; (4) the "maltose" figure did not change in the higher grades. Baker and Hulton (1908) found that diastatic activity increased during storage of flour (reported by Bailey, 1925). Hartmann (1930) stored unbleached flour for fifteen months and concluded that the gluten nitrogen decreases on storage at a rapid rate, while Barton-Wright

(1938) reports an increase in soluble nitrogen after ten weeks of storage.

Marotta, Di Stefano, and Vercillo (1935) studied the effect of aging on the diastatic value of flour. They found that neither the diastase value nor the amount of reducing sugar changes during aging.

No mention has been made of the extensive work done on the effect of storage on hydrogen-ion concentration, total acidity, or on the fatty components of flour, since these factors were not considered in the analyses reported in this paper.

### Experimental

Two different chemically bleached and matured flours were chosen for this study, the flours being considered as representative bakers' flours. One sample was milled from new wheat only, while the percent of new wheat in the other was not known. Both were high-grade patent flours. The kinds of bags used were paper-lined, jute, grain, and cotton. About 30 lbs. of each sample of flour were stored in each of the four different kinds of bags. Two sets of samples were prepared. One set of samples in each kind of bag was used for weighing at regular intervals, while the other was used for analysis and baking tests. The flour, the sample number, and the kind of bag are shown in Table I. The samples of flour in the various bags will be referred to in later

TABLE I  
SAMPLES OF FLOUR AS THEY WERE STORED IN DIFFERENT BAGS

Flour	Sample number	Kind of bag
High-grade chemically bleached and matured patent from all new wheat. Aged normal time at mill before receipt on Nov. 17, 1937.	1B	Paper-lined
	2B	Jute
	3B	Grain
	4B	Cotton
High grade chemically bleached and matured patent. Amount of new wheat unknown. Aged normal time before receipt on Sept. 30, 1937.	5B	Grain
	6B	Jute
	7B	Paper-lined
	8B	Cotton

tables by the sample numbers given in Table I. At regular intervals the flour samples were analyzed for moisture, absorption, ash, and protein; and baking tests were made.

The two different flours were sampled on November 17, 1937. The first analyses and baking tests were made on this date. The recordings of weights, temperatures, and humidity of the storage room were started on November 18th. The flour samples were stored in a dry, clean room in the basement which closely approached average bake-shop conditions. The weight of the bags and the temperature and



humidity of the storage room were recorded daily (data are missing for week-ends and a few other days).

In order to have a comparison of the weights on a commercial scale a skid of the flour in cotton bags (15 bags) used for samples 5B, 6B, 7B, and 8B was placed in a storage room of a commercial bakery and the temperature, humidity, and weight recorded weekly.

The tests were started on the flour used for samples 1B, 2B, 3B, and 4B when it had been aged about as much as is usual when the baker receives it; the other sample was about 45 days older.

All absorptions were determined by mixing flour and water in a Fleischman mixer. One hundred grams of flour were used and water was run in from a burette during mixing until a dough of a standard consistency was reached. This standard consistency was determined by the feel of an experienced operator. The same operator determined the absorptions in all cases. From experiments the absorptions are correct at least to  $\pm 0.5$  percent.

The methods used for determining moisture,<sup>1</sup> ash, and protein ( $N \times 5.7$ ) were those of the Association of Official Agricultural Chemists. All volumes were determined by the method described by Cathcart and Cole (1938).

Both laboratory and commercial baking tests were performed. The laboratory test was made in a tall-form pan without the aid of enriching ingredients. It was made in two parts, the second part providing 45 minutes more fermentation time than the first part. This gave an indication of fermentation tolerance and general baking characteristics. However, in order to obtain a truer picture of how the flour would behave in a bake shop a commercial loaf was baked. This loaf contained enriching ingredients, yeast food, and was a regular one-pound, round-top loaf of bread. The straight-dough method of test baking was used throughout. Data from the two parts of the laboratory loaves were scored under the headings of fermentation tolerance, oven spring, bread quality, aroma and taste, and color of crumb. Each factor was rated as shown in Table II. Each part of the laboratory baking test was measured for volume and photographs were made.

The commercial loaves were scored for volume, color of crust, symmetry of form, evenness of bake, character of crust, break and shred, grain, color of crumb, aroma, taste, and texture, numerical figures being given in each case.

## Results

The data for moisture, absorption, and the baking tests for samples 1B, 2B, 3B, 4B, 5B, 6B, 7B, and 8B are recorded in Table II. The

<sup>1</sup>Air-oven method.

TABLE II

DATA ON FLOUR SAMPLES STORED IN VARIOUS BAGS, GIVEN ACCORDING TO THE KIND OF BAG

VG = Very Good, G = Good, FG = Fairly Good, F = Fair, P = Poor, CW = Creamy White, W = White, GW = Gray White, DG = Dark Gray, and GR = Gray.

Sample No. and kind of bag	Date tested	Mois- ture as re- ceived, %	Ab- sor- ption on 15% mois- ture basis, %	Laboratory baking-test data						Commercial baking-test data							
				Volume, cu. in./oz.		Toler- ance	Oven spring	Bread quality	Aroma and taste	Color of crumb	Vol., cu. in./ oz.	Grain, 10	Color of crumb, 10	Aroma, 15	Taste, 20	Tex- ture, 15	Total score, 100
				1st part	2nd part												
1B, Paper lined	Nov. 17, 1937	11.4	57.3	9.7	9.5	VG	VG	VG	VG	GW	9.8	9.0	9.5	14.0	19	14.0	93.5
	Dec. 1, 1937	11.4	57.8	9.6	10.1	VG	VG	VG	VG	CW	10.7	9.0	9.0	14.0	19	14.0	93.0
	Dec. 15, 1937	11.2	57.9	9.5	9.7	VG	VG	VG	VG	CW	9.2	8.5	9.0	14.0	19	13.0	91.0
	Jan. 5, 1938	11.0	58.5	9.1	8.6	VG	G	G	G	GW	8.3	8.0	8.0	13.5	19	14.0	91.0
	Jan. 26, 1938	10.4	58.0	9.4	8.4	VG	VG	VG	VG	CW	10.0	9.0	9.0	14.0	19	14.0	93.5
	Feb. 16, 1938	10.1	57.4	9.7	9.5	VG	VG	VG	VG	CW	10.2	9.0	9.0	14.0	19	14.0	93.5
	Mar. 9, 1938	9.8	57.3	9.0	8.4	G	VG	VG	VG	GW	10.0	9.0	9.0	14.0	19	14.0	93.0
	April 13, 1938	10.0	58.2	9.1	9.4	G	G	G	G	VG	10.0	9.0	8.5	14.0	19	14.0	92.5
	May 6, 1938	9.9	58.1	8.8	8.2	G	G	F	FG	GR	9.4	8.5	8.0	13.5	19	13.5	90.0
	June 2, 1938	10.8	59.1	8.8	8.5	G	G	FG	P	GR	9.4	8.0	8.0	12.0	15	13.5	83.5
2B, Jute	Nov. 17, 1937	11.7	57.9	9.3	9.4	VG	VG	VG	VG	VG	9.2	9.0	9.5	14.0	19	14.0	93.5
	Dec. 1, 1937	11.3	57.6	9.6	9.4	VG	VG	VG	VG	CW	10.8	9.0	9.0	14.0	19	14.0	93.0
	Dec. 15, 1937	11.4	58.3	9.5	9.5	VG	VG	VG	VG	CW	9.4	8.5	9.0	14.0	19	13.0	91.0
	Jan. 5, 1938	10.9	58.4	9.2	8.3	VG	VG	VG	VG	G	8.4	8.0	8.5	13.5	19	14.0	91.0
	Jan. 26, 1938	10.1	57.4	8.6	8.3	VG	VG	VG	VG	CW	9.7	9.0	9.0	14.0	19	14.0	93.5
	Feb. 16, 1938	9.9	57.1	9.4	9.7	VG	VG	VG	VG	CW	10.4	9.0	9.0	14.0	19	14.0	93.5
	Mar. 9, 1938	9.5	56.9	8.6	9.2	G	VG	VG	VG	GW	9.5	9.0	9.0	14.0	19	14.0	93.0
	April 13, 1938	10.2	58.5	9.3	9.3	G	G	G	G	VG	9.8	9.0	8.5	14.0	19	14.0	92.5
	May 6, 1938	10.1	58.4	7.8	7.5	F	P	P	F	DG	9.5	8.5	8.0	13.5	19	13.0	89.5
	June 2, 1938	11.1	59.7	8.4	8.6	FG	FG	FG	P	GR	9.6	7.5	8.0	12.0	15	13.0	82.5

TABLE II—Continued

Sample No. and kind of bag	Date tested	Mois- ture as re- ceived, %	Ab- sorp- tion on 15% mois- ture basis, %	Laboratory baking-test data						Commercial baking-test data							
				Volume, cu. in./oz.	Toler- ance	Oven spring	Bread quality	Aroma and taste	Color of crumb	Vol., cu. in./ oz.	Grain, 10	Color of crumb, 10	Aroma, 15	Taste, 20	Tex- ture, 15	Total score, 100	
BBB, Grain	Nov. 17, 1937	11.4	57.3	9.6	9.1	VG	VG	VG	VG	GW	10.0	9.0	9.5	14.0	19	14.0	93.5
	Dec. 1, 1937	11.4	57.8	9.7	9.3	VG	VG	VG	VG	CW	10.4	9.0	9.0	14.0	19	14.0	93.0
	Dec. 15, 1937	11.2	57.9	9.5	9.5	VG	VG	VG	G	CW	10.2	8.5	9.0	14.0	19	13.5	91.5
	Jan. 5, 1938	10.9	58.4	8.9	8.8	VG	G	VG	G	GW	8.4	8.5	8.5	13.5	19	14.0	93.0
	Jan. 26, 1938	10.3	57.8	9.1	8.6	VG	VG	VG	VG	CW	9.8	9.5	9.0	14.0	19	14.0	93.5
	Feb. 16, 1938	9.9	57.1	9.4	9.5	VG	VG	VG	VG	CW	10.4	9.0	9.0	14.0	19	14.0	93.5
	Mar. 9, 1938	9.9	57.5	9.6	8.6	G	VG	VG	VG	CW	9.9	9.0	9.0	14.0	19	14.0	93.0
	April 13, 1938	10.0	58.2	9.4	9.5	G	G	G	VG	GW	9.9	9.0	8.5	14.0	19	14.0	92.5
	May 6, 1938	10.2	58.5	8.7	9.1	G	F	FG	G	GW	9.3	8.5	8.5	13.5	19	13.5	90.0
	June 2, 1938	11.1	59.7	8.6	9.0	G	G	FG	F	GW	9.3	7.5	8.0	12.0	15	13.5	83.0
4BB, Cotton	Nov. 17, 1937	12.1	58.6	9.5	9.4	VG	VG	VG	VG	VG	10.7	9.0	9.5	14.0	19	14.0	93.5
	Dec. 1, 1937	11.9	58.7	9.5	9.3	VG	VG	VG	VG	CW	10.6	9.0	9.0	14.0	19	14.0	93.0
	Dec. 15, 1937	11.7	58.8	9.6	9.5	VG	VG	VG	G	CW	10.3	9.0	9.0	14.0	19	14.0	93.0
	Jan. 5, 1938	10.9	58.4	9.0	8.5	VG	G	VG	G	CW	8.4	8.5	8.5	13.5	19	14.0	91.5
	Jan. 26, 1938	10.5	58.1	9.0	9.0	VG	VG	VG	VG	GW	10.0	9.5	9.0	14.0	19	14.0	94.0
	Feb. 16, 1938	10.0	57.2	9.6	9.4	VG	VG	VG	VG	CW	10.3	9.0	9.0	14.0	19	14.0	93.5
	Mar. 9, 1938	9.7	57.2	9.5	8.9	G	VG	VG	VG	GW	10.0	9.0	9.0	14.0	19	14.0	93.0
	April 13, 1938	10.2	58.5	9.1	9.1	G	G	VG	G	GW	9.5	9.0	8.5	14.0	19	14.0	92.5
	May 6, 1938	10.3	58.7	8.5	8.9	FG	FG	FG	FG	GW	9.0	8.5	8.5	13.5	19	14.0	90.5
	June 2, 1938	11.0	59.5	8.5	8.8	FG	FG	FG	FG	GW	9.0	7.5	8.0	12.0	15	13.5	83.0

TABLE II—Continued

Sample No. and kind of bag	Date tested	Mois- ture as re- ceived, %	Ab- sor- tion on 15% mois- ture basis, %	Laboratory baking-test data						Commercial baking-test data							
				Volume, cu. in./oz.		Toler- ance	Oven spring	Bread quality	Aroma and taste	Color of crumb	Vol., cu. in./ oz.	Grain, 10	Color of crumb, 10	Aroma, 15	Taste, 20	Tex- ture, 15	Total score, 100
				1st part	2nd part												
5B, Grain	Nov. 17, 1937	11.8	57.1	9.3	9.2	VG	VG	VG	VG	CW	10.1	9.5	9.5	14.0	19	14.0	94.0
	Dec. 1, 1937	11.8	58.0	9.2	9.3	VG	VG	VG	VG	CW	10.9	9.0	9.5	14.0	19	14.0	93.0
	Dec. 15, 1937	11.6	58.2	10.0	9.7	VG	VG	VG	VG	CW	10.3	9.5	9.5	14.0	19	14.0	94.5
	Jan. 5, 1938	11.1	58.2	9.5	8.9	VG	VG	VG	VG	CW	8.4	9.0	9.0	14.0	19	14.5	93.5
	Jan. 26, 1938	10.4	57.5	9.2	9.3	VG	VG	VG	VG	CW	10.1	9.5	9.0	14.0	19	14.0	94.0
	Feb. 16, 1938	10.1	57.0	9.7	9.3	VG	VG	VG	VG	W	10.6	9.0	9.5	14.0	19	14.0	93.5
	Mar. 9, 1938	9.8	56.8	9.5	9.5	VG	VG	VG	VG	W	9.9	9.0	9.5	14.0	19	14.0	94.0
	April 13, 1938	10.4	58.4	9.5	9.3	VG	VG	VG	VG	CW	9.8	9.0	9.0	14.0	19	14.0	93.5
	May 6, 1938	10.3	58.2	8.7	8.8	VG	G	G	G	W	10.1	9.0	9.0	14.0	19	14.0	93.5
	June 2, 1938	11.0	59.0	9.5	8.5	G	G	FG	F	W	9.1	8.5	8.5	12.0	15	14.0	85.0
	Nov. 17, 1937	12.3	58.0	10.0	9.4	VG	VG	VG	VG	CW	9.4	9.5	9.5	14.0	19	14.0	94.0
	Dec. 1, 1937	12.0	58.4	9.7	9.4	VG	VG	VG	VG	CW	10.6	9.0	9.5	14.0	19	14.0	93.5
6B, Jute	Dec. 15, 1937	11.8	58.5	10.2	9.5	VG	VG	VG	VG	CW	9.9	9.0	9.5	14.0	19	14.0	94.0
	Jan. 5, 1938	11.2	58.4	9.6	9.0	VG	VG	VG	VG	CW	8.6	9.5	9.0	14.0	19	14.5	94.0
	Jan. 26, 1938	10.8	58.2	8.8	9.8	VG	VG	VG	VG	CW	10.0	9.0	9.0	14.0	19	14.0	93.5
	Feb. 16, 1938	10.2	57.1	9.8	9.4	VG	VG	VG	VG	W	10.4	9.0	9.5	14.0	19	14.0	93.5
	Mar. 9, 1938	10.1	57.4	9.3	9.6	VG	VG	VG	VG	W	9.0	9.0	9.5	14.0	19	14.0	94.0
	April 13, 1938	10.4	58.4	9.6	9.4	VG	VG	VG	VG	CW	9.8	9.0	9.0	14.0	19	14.0	93.5
	May 6, 1938	10.1	57.9	8.9	9.1	VG	G	G	G	W	10.2	9.5	9.0	14.0	19	14.0	94.0
	June 2, 1938	10.9	58.8	9.7	8.4	G	G	FG	F	W	9.4	8.0	8.5	12.0	15	14.0	84.5

TABLE II—Continued

Sample No., and kind of bag	Date tested	Moisture as re- ceived, %	Ab- sorp- tion on 15% mois- ture base, %	Laboratory baking-test data						Commercial baking-test data								
				Volume, cu. in./oz.		Toler- ance	Oven spring	Bread quality	Aroma and taste	Color of crumb	Vol., cu. in./ oz.	Grain, 10	Color of crumb, 10	Aroma, 15	Taste, 20	Tex- ture, 15	Total score, 100	
				1st part	2nd part													
7B, Paper lined	Nov. 17, 1937	12.1	57.6	9.8	9.8	VG	VG	VG	VG	CW	CW	9.9	9.5	9.5	14.0	19	14.0	94.0
	Dec. 1, 1937	12.0	58.4	9.7	9.5	VG	VG	VG	VG	CW	CW	10.5	9.0	9.5	14.0	19	14.0	93.5
	Dec. 15, 1937	11.8	58.5	10.6	9.5	VG	VG	VG	VG	CW	CW	10.3	9.0	9.5	14.0	19	14.0	94.0
	Jan. 5, 1938	11.2	58.4	9.4	9.0	VG	VG	VG	VG	CW	CW	8.4	9.0	9.0	14.0	19	14.5	93.5
	Jan. 26, 1938	10.4	57.5	9.2	9.5	VG	VG	VG	VG	CW	CW	10.9	9.0	9.0	14.0	19	14.0	93.0
	Feb. 16, 1938	10.4	57.5	9.4	9.7	VG	VG	VG	VG	W	W	10.3	9.0	9.5	14.0	19	14.0	93.5
	Mar. 9, 1938	10.2	57.6	9.5	9.3	VG	VG	VG	VG	W	W	10.0	9.0	9.5	14.0	19	14.0	94.0
	April 13, 1938	10.4	58.4	9.5	9.2	VG	VG	VG	VG	CW	CW	9.8	9.0	9.0	14.0	19	14.0	93.5
	May 6, 1938	10.1	57.9	8.9	8.6	VG	VG	G	VG	CW	CW	9.3	9.5	9.0	14.0	19	14.0	94.0
	June 2, 1938	10.9	58.8	9.5	8.2	G	VG	G	FG	CW	CW	9.7	8.5	8.5	12.0	15	14.0	84.5
8B, Cotton	Nov. 17, 1937	11.6	56.7	9.7	9.6	VG	VG	VG	VG	VG	CW	10.2	8.5	9.5	14.0	19	14.0	94.0
	Dec. 1, 1937	11.7	57.9	9.9	9.7	VG	VG	VG	VG	CW	CW	10.5	9.0	9.5	14.0	19	14.0	93.5
	Dec. 15, 1937	11.7	58.4	10.8	9.3	VG	VG	VG	VG	CW	CW	10.1	9.5	9.5	14.0	19	14.0	94.5
	Jan. 5, 1938	11.2	58.4	9.5	9.1	VG	VG	VG	VG	CW	CW	8.4	9.0	9.0	14.0	19	14.5	93.5
	Jan. 26, 1938	10.4	57.5	9.1	9.8	VG	VG	VG	VG	CW	CW	10.8	9.0	9.0	14.0	19	14.0	93.0
	Feb. 16, 1938	10.6	57.8	9.5	9.5	VG	VG	VG	VG	W	W	10.3	9.0	9.5	14.0	19	14.0	93.5
	Mar. 9, 1938	10.1	57.4	9.7	9.5	VG	VG	VG	VG	W	W	11.0	9.0	9.5	14.0	19	14.0	94.0
	April 13, 1938	10.4	58.4	9.8	8.6	VG	VG	VG	VG	CW	CW	10.5	9.0	9.0	14.0	19	14.0	93.5
	May 6, 1938	10.1	57.9	9.3	9.1	VG	VG	G	VG	CW	CW	10.0	9.5	9.0	14.0	19	14.0	94.0
	June 2, 1938	11.0	59.0	9.3	8.8	G	VG	G	FG	CW	CW	9.0	8.5	8.5	12.0	15	14.0	84.0

data are recorded according to the kind of bag used for storage. In order to conserve space all of the factors scored on the commercial loaf are not given; only those which are considered to be the most important are presented.

The data on the weights of the various bags of the samples and the humidities and temperatures of the storage room are plotted in Figure 1. Similar data for the skid of commercial flour are given in Figure 2, beginning immediately upon receipt of the flour. The data for absorption of all samples obtained on November 17, 1937, March 9, 1938, and June 2, 1938, are summarized in Table III.

Photographs of the laboratory loaves made on February 16, 1938, while the flour still retained its optimum qualities, are given in Figure 3. Those made on June 2, after the flour had deteriorated in quality with respect to some factors, are given in Figure 4.

TABLE III

DATA SHOWING CHANGE IN ABSORPTION ON SAMPLES IN VARIOUS BAGS FROM NOVEMBER 17, 1937, TO MARCH 9, 1938, AND TO JUNE 2, 1938

Sample number	Kind of bag	Date	Absorption as received, %	Absorption calculated from moisture, %	Moisture, %	Absorption on 15% moisture basis, %
1B	Paper-lined	Nov. 17, 1937	64.0		11.4	57.3
		Mar. 9, 1938	67.0	66.9	9.8	57.3
		June 2, 1938	67.0	65.1	10.8	59.1
2B	Jute	Nov. 17, 1937	64.0		11.7	57.9
		Mar. 9, 1938	67.0	68.1	9.5	56.9
		June 2, 1938	67.0	65.1	11.1	59.7
3B	Grain	Nov. 17, 1937	64.0		11.4	57.3
		Mar. 9, 1938	67.0	66.8	9.9	57.5
		June 2, 1938	67.0	64.5	11.1	59.7
4B	Cotton	Nov. 17, 1937	64.0		12.1	58.6
		Mar. 9, 1938	67.0	68.5	9.7	57.2
		June 2, 1938	67.0	66.1	11.0	59.5
5B	Grain	Nov. 17, 1937	63.0		11.8	57.1
		Mar. 9, 1938	66.5	66.7	9.8	56.8
		June 2, 1938	66.5	64.5	11.0	59.0
6B	Jute	Nov. 17, 1937	63.0		12.3	58.0
		Mar. 9, 1938	66.5	67.1	10.1	57.4
		June 2, 1938	66.5	65.6	10.9	58.8
7B	Paper-lined	Nov. 17, 1937	63.0		12.1	57.6
		Mar. 9, 1938	66.5	66.5	10.2	57.6
		June 2, 1938	66.5	65.2	10.9	58.8
8B	Cotton	Nov. 17, 1937	63.0		11.6	56.7
		Mar. 9, 1938	66.5	65.7	10.1	57.4
		June 2, 1938	66.5	64.1	11.0	59.0

### Discussion

The moisture content of all the samples at the beginning of the test were normal, ranging from 11.4 to 12.3 percent. They all decreased to a minimum during the fourth month and then started increasing. At the end of the test, that is, at the end of approximately seven months, they were practically back to the moisture content at which they had started. The kind of bag had little effect on the variation in moisture content.

The percentages of ash and protein showed no significant change in any of the samples. Apparently all of the variations noted were due to experimental error. It is concluded that over the period of storage the ash and protein content of both flours in all bags remained the same, which is in agreement with the results reported by Kent-Jones (1924).

Values for absorption showed considerable variation. However, the absorption for all samples in all of the various bags showed an increase at the end of the experiment over that at the beginning. This is in harmony with the work of other investigators cited in the introduction to this paper. After about the third month, when the moisture content had reached its low point, absorptions for samples 1B in a paper-lined bag, 7B in a paper-lined bag, 3B in a grain bag, and 5B in a grain bag showed that the flour took up all of the water in mixing that it had lost as a result of evaporation. Sample 2B in a jute bag and sample 6B in a jute bag showed a decrease at this point. They did not take up all of the moisture which they had lost by evaporation. Sample 4B in a cotton bag showed this same decrease, while sample 8B in a cotton bag gave opposite results, showing an increase at this point. These data on absorption have been summarized in Table III. Absorptions on a 15% moisture basis, as well as calculated absorptions dependent upon the change in moisture and the absorption as received, are given.

The baking tests showed definitely that there was a difference in the two different flours used, yet in no case is there any significant difference in the same flour sample when stored in the various bags. The flour samples stored in the various bags with sample numbers 1B, 2B, 3B, and 4B began to show deterioration in baking quality on March 9, 1938, as is evident from the laboratory baking tests. The quality of the commercial bread containing enriching ingredients, however, held up for approximately two months longer. Infestation became evident May 6, 1938. The flour stored in the various bags with sample numbers 5B, 6B, 7B, and 8B, did not show deterioration in the laboratory baking tests until May 6. The commercial score at this date was very good. The next month the commercial score showed quite a decrease, however. The decrease in quality immediately after

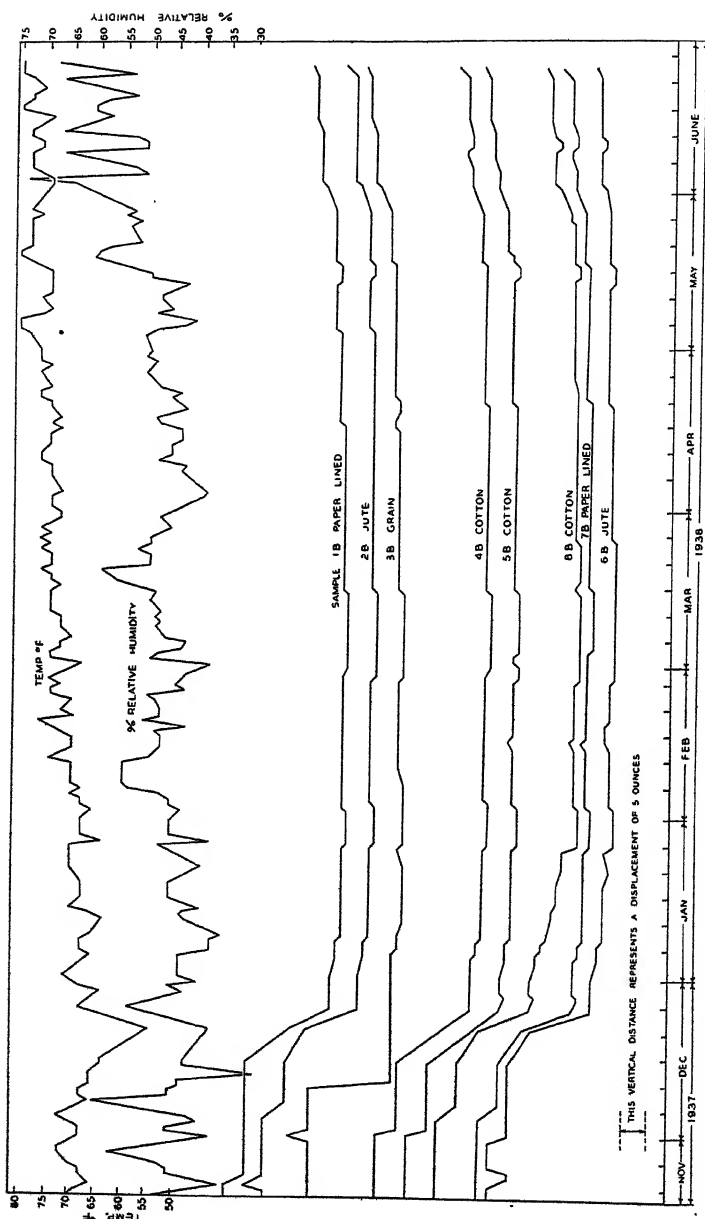


Fig. 1. Weight changes in ounces of the various samples of flour during storage. Changes in temperature and relative humidity of the storage room are also shown.



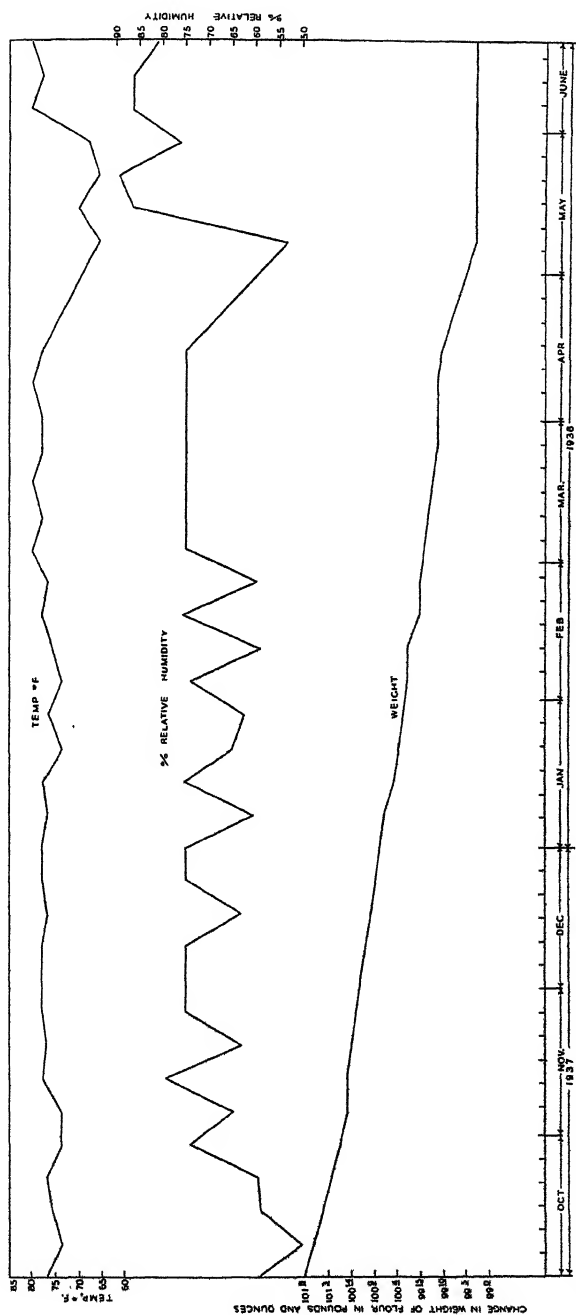


Fig. 2. Weight changes in ounces per bag of a skid of flour during storage in a bakery. The changes in temperature and relative humidity of the storage room are also shown.

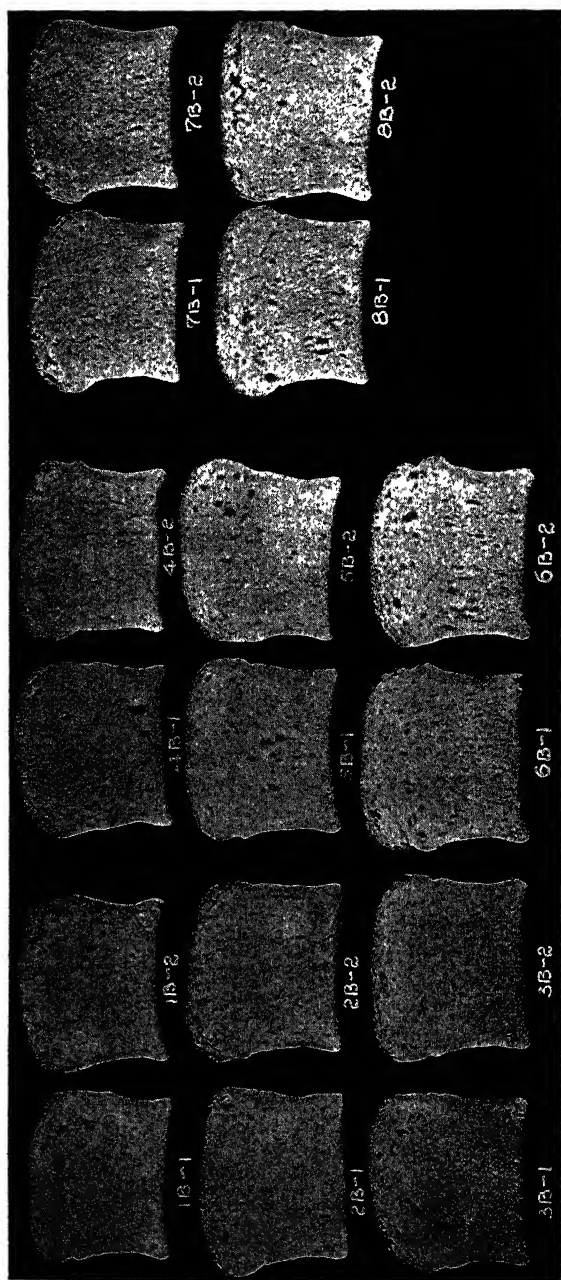


Fig. 3. Laboratory loaves made on February 16, 1938, given by sample number. The "1" following the dash represents first part of laboratory bake; the "2" represents the second part. All samples were as good on this date as at beginning of the test.

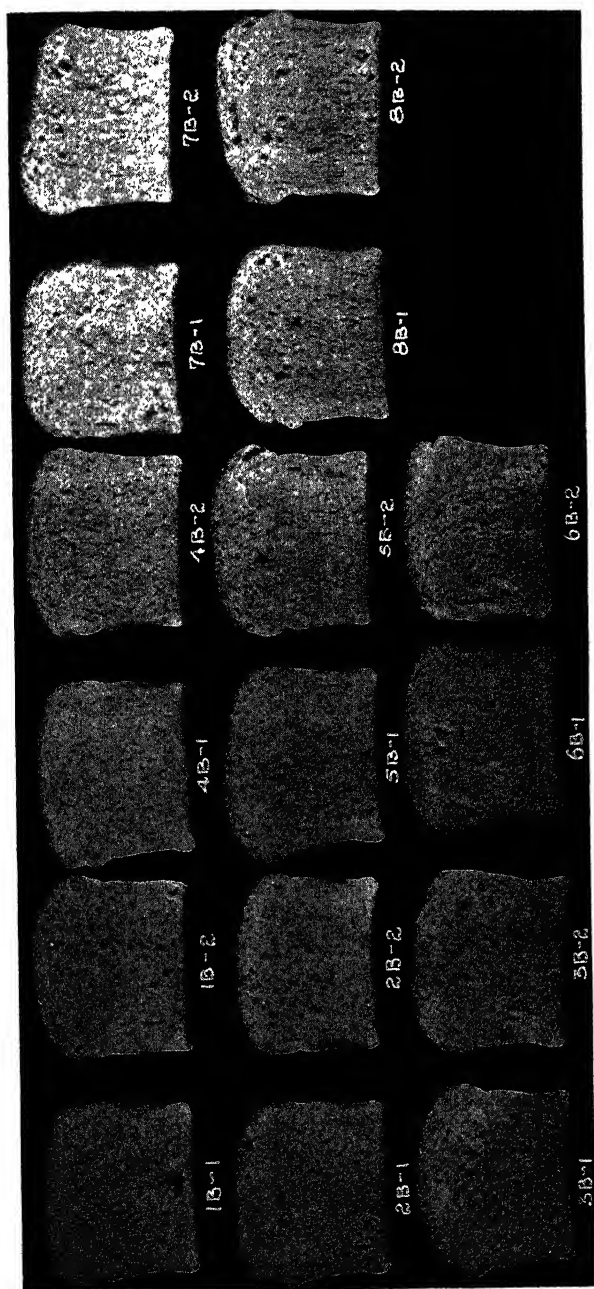


Fig. 4. Laboratory loaves made on June 2, 1938, given by sample numbers. The "1" following the dash represents first part of laboratory bake; the "2" represents the second part. Practically all characteristics shown by the photographs are as good as on February 16, 1938; however, the total scores are lower mainly because of aroma, taste, and color.

the baking tests on May 6 is due to the fact that infestation had developed since the previous test. Thus the baking test on June 2 is of little significance, except to show the great decrease in quality. From an examination of Table II, it is evident from the baking tests of all samples down to May 6 that there was no difference in the baking quality of each separate flour which was stored in the various bags. Although the baking tests after this show some variation, it is doubtful that this was due to the infestation. However, the bakes on May 6 and June 2 show a slight advantage, perhaps, for the paper and cotton bags.

The fact that both flours had maximum baking quality at the beginning of the experiments and after a time showed a decrease agrees with the work of Kuhl and Kliefoth (1934). As far as could be determined by the baking tests, the diastatic activity of the flours showed little change during storage, which is in agreement with the work of Kent-Jones (1924) and Marotta, Di Stefano and Vercillo (1935).

Photographs of all the bakes could not be given; however, the laboratory loaves made on February 16, 1938, before any of the samples showed any deteriorations, are shown in Figure 3, and the corresponding loaves made on the last test June 2, 1938, are given in Figure 4. It is evident from these pictures and the data of Table II, that the deterioration in the samples is mainly due to degradation of the aroma, taste, and color and not to the other factors of the score.

The volume on various days showed considerable variation and was quite low on January 5 in the case of the commercial samples. The only explanation which can be given for this is the uncontrollable personal factor of the operator. On the whole, the results are quite consistent and there is no significant difference in the volumes from the samples in the various bags. Nearly all samples showed just about as high a volume at the end of the test as they did in the beginning. This is especially true of the commercial loaves and is in agreement with work of Swanson, Willard, and Fitz (1915), Saunders, Nichols and Cowan (1921), and Greaves (1926).

The weights on the various bags are given in Figure 1 along with the temperature and humidity of the storage room. The samples which were weighed contained approximately 30 lbs. of flour, and up to December 24, 1937 the bags were only weighed to the nearest quarter pound. However, at this time the scale was changed and the bags weighed from this point on to the nearest ounce. Thus, in examining the results it is suggested that the weights given before December 28 be disregarded. It will be noted that the weights vary approximately the same in all bags. All of the curves decrease to a minimum and then increase. The curves as given are not actual weights but the varia-

tions from the first weight. From these tests the kind of bag has little effect on the rate of moisture loss or on the rate of gain in moisture. Thus, the results do not verify Krtinsky's (1937) results that jute allows a greater rate of moisture loss than paper bags. It will be noted from Figure 1 that there was considerable variation in temperature and humidity throughout the period of storage.

Figure 2 is of interest only for comparison. The kind of bag used for the flour was ordinary cotton. Although there was considerable variation in humidity and temperature during the storage period, the flour showed a gradual decrease in weight throughout the storage period. In other words, the variation in humidity and temperature did not cause fluctuation in the weight of the flour per bag as was noted with the smaller samples. This is what is to be expected, however, from the results of Arpin and Pecaud (1923). On April 14, 1938 the storage room of the skid of flour was changed and for a period of approximately three weeks no records were made.

### Conclusions

Of the two chemically matured and bleached flours used in this test, one held up longer during storage than the other. Both had optimum baking qualities at the beginning of the test. One flour did not begin to deteriorate until about the fourth month, the other flour until about the sixth month. Deterioration in the latter sample seemed to be due mainly to insect infestation. As far as could be determined from the analyses, there was no change in the protein or ash content of these samples during storage. The absorption showed an increase in all cases. The kind of bag used for storage made little difference in the results of the analyses or baking tests, with the possible exception of absorption. In a few cases near the half-way point the flour did not regain all of the moisture which had been lost during storage. It is hoped that the effect of the various bags on absorption can be re-studied with a more accurate measure for absorption.

### Summary

Two different samples of chemically bleached and matured flour were stored separately in four different types of bags, namely ordinary cotton, paper-lined, jute, and grain. They were stored under average commercial bake-shop conditions. The samples were analyzed at regular intervals for moisture, ash, protein, and absorption, and baking tests were made. The kind of bag had little effect on the analyses or the baking quality of the flour. In all cases absorption increased during storage, ash and protein showed no change, and the baking quality in one sample fell off after the fourth month and in the other

after the sixth month. Deterioration in the latter case was due to infestation. The effects incidental to storage are in general agreement with the work of other investigators.

### Acknowledgment

The help of Richard Ryberg with the analyses and Steven Luber and Andrew Habenicht with the baking tests is gratefully acknowledged. The authors also wish to thank a member of the American Bakers' Association for the data reported in Figure 2, for taking the photographs, and for supplying the various bags.

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## GAS RETENTION AS AFFECTED BY INCLUDING SHORTENING IN THE TENTATIVE A.A.C.C. BASIC TEST-BAKE FORMULA<sup>1</sup>

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(Read at the Annual Meeting, May 1939)

In 1937 the author reported that shortening used in a straight-dough commercial formula, up to 4%, increased loaf volume slightly, with all classes of shortening tested, and that shortening lowered the height of the gas retention curve.<sup>2</sup>

The tests were made with a commercial formula. The baking committee decided to study the effect of shortening on gas retention, using the A.A.C.C. basic procedure. A study of this nature seemed to be desirable since some of the members using the A.A.C.C. basic procedure include shortening in their formula.

### Experimental

Three extractions of bakers' flour (patent, straight, and first clear bleached and unbleached) were selected for this test. Preliminary tests were performed to determine the absorption and approximate mixing time. Two levels of shortening were used, namely 2% and 4%, imposed on the A.A.C.C. basic procedure with absorption and mixing time predetermined. From the same piece of dough on which the gas-production and gas-retention tests were made, duplicate baking tests were also made at 86° temperature for fermentation and proof, with the 3-hour fermentation as called for in the basic procedure.

<sup>1</sup> Sub-committee report, 1938-39 Committee on Standardization of Laboratory Baking.

<sup>2</sup> W. L. Heald, Effect of different types of shortening on white pan bread, *Cereal Chem.* 14: 481-488, 1937.

Table I shows the results obtained on the three flours which were commercially bleached and diastatically treated. The first column shows that the total gas on all flours was slightly reduced by the addition of shortening. This has been verified by a number of methods other than gas retention. Under "Gas retained," we find that in the case of the patent and straight flours, the 2% and 4% of shortening definitely reduce the percent of gas retained over the 6-hour fermentation period. In the clear flour a different result is obtained. Here we find that 2% of shortening definitely increases gas retention and that 4% gives the same results as no shortening at all.

TABLE I

EFFECT OF SHORTENING ON GAS RETENTION AND ON LOAF VOLUME FOR BLEACHED FLOURS

	Rate per hour of total gas	Gas retained	Time to elastic limit	Volume
	cc./100 fl.	%	min.	cc.
PATENT (BLEACHED)				
No shortening	386	38.7	291	535
2% "	376	34.0	242	524
4% "	374	34.1	263	530
STRAIGHT (BLEACHED)				
No shortening	394	41.0	304	556
2% "	405	34.3	238	597
4% "	385	34.0	228	587
CLEAR (BLEACHED)				
No shortening	422	31.0	215	510
2% "	420	34.7	268	554
4% "	405	30.9	218	585

The upper curve (Fig. 1), representing 2% shortening, is perfectly smooth until it reaches its elastic limit. In the lower curve there is a break which definitely shows a reduction in gas retention up to a certain point, when a gradual rise in gas retained occurs. This explains why the percentage of gas retained is slightly greater in the clear flour with the shortening than with no shortening whatsoever.

We have found in some of our experiments that shortening has a tendency to mellow a slightly bucky dough. Wherever the flour has dropped on the gas-retention curve and then started to retain gas again the result was a rather bucky dough. Shortening apparently eliminates this; if not entirely it at least helps.

In the next column, "Time to elastic limit," we find that again in the patent and straight flours the time is reduced considerably to the elastic limit, but in the bleached clear flour the 2% shortening increased the time to this point. The unbleached clear also showed less time to the limit with both 2% and 4% shortening.



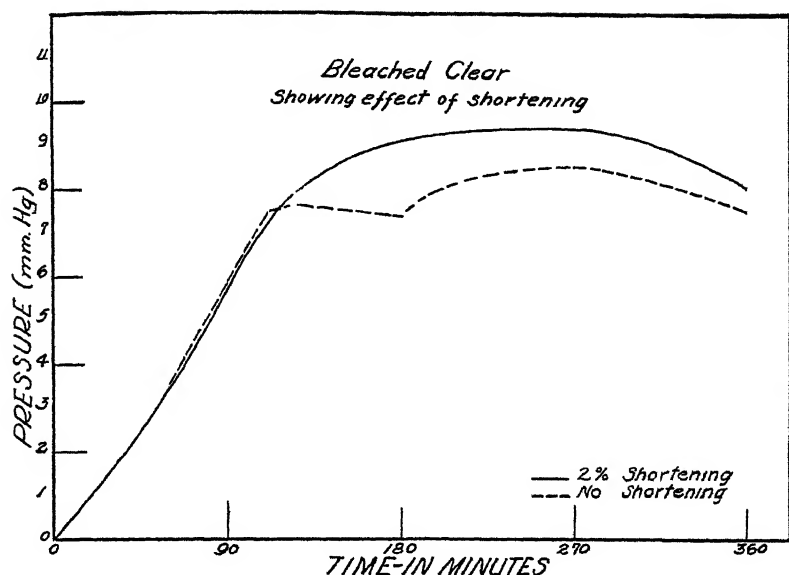


Figure 1.

In the "Volume" column it appears that the effect of shortening on volume for the patent flour was practically negligible with slight increase in volume for straight grades and clears.

Table II carries out the same idea as Table I, except that unbleached flours are used in place of bleached flours. We find that in

TABLE II

EFFECT OF SHORTENING ON GAS RETENTION AND LOAF VOLUME FOR UNBLEACHED FLOURS

	Rate per hour of total gas	Gas retained	Time to elastic limit	Volume
	cc./100 fl.	%	min.	cc.
PATENT (UNBLEACHED)				
No shortening	400	36.4	268	562
2% "	390	32.4	217	560
4% "	364	34.8	214	573
STRAIGHT (UNBLEACHED)				
No Shortening	375	37.1	247	539
2% "	372	31.6	184	542
4% "	360	29.1	209	562
CLEAR (UNBLEACHED)				
No Shortening	417	28.8	235	518
2% "	387	29.5	222	551
4% "	373	29.4	199	562

general the same results are obtained as with the bleached flours, with the exception of unbleached clear.

### Summary of Results

It may be concluded that with the apparatus used and with the patent and straight-grade flours, bleached and unbleached, gas retention (as determined in percent of total gas retained in the dough) was reduced by the use of shortening. While in the bleached clear flour 2% shortening had a tendency to increase gas retention, 4% shortening did not materially change the percent of gas retained over the 2%.

In all cases of patent, straight, and clear flours the total rate of gas per hour was slightly reduced. In the case of the patent and straight grades the time to the elastic limit was also reduced. In the clear bleached flour the time to the elastic limit was increased, while in the unbleached it was reduced quite materially.

It is our belief that the inclusion of shortening in the A.A.C.C. formula would tend to make the baking results more satisfactory on a flour that exhibits bucky characteristics. However, the tendency to mask this bucky effect might render the test bakes less useful. In spite of this fact and knowing that the baker uses a rather liberal amount of shortening in his formula, I would recommend 3% shortening as being a very desirable amount to use in the A.A.C.C. baking formula.

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## REPORT OF THE 1938-39 A.A.C.C. COMMITTEE ON STANDARDIZATION OF LABORATORY BAKING<sup>1</sup>

CLAUDE F. DAVIS, *Chairman*

Western Star Mill Co., Salina, Kansas

(Read at the Annual Meeting, May 1939)

During the past two years the baking committee has attempted to encourage the development of test-bake equipment with the idea that some outstanding types would be acceptable for standardizing the test and that such equipment would become readily available to many laboratories and materially overcome some of the test-bake problems. Some of the problems are high experimental error in loaf volume, double breaks in oven expansion, high loaf ends, more uniformity of inside and outside loaf characteristics between replicates in the same bake, more consistent results from day to day, and the personal factor in dough manipulation.

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<sup>1</sup> General report.

With these problems more nearly under control we would be in better position to determine flour characteristics as shown by the basic and all supplementary treatments within and between different laboratories. With the complex system dealt with in test baking one cannot be surprised at the rather high experimental error that persists in spite of all precautions. If this error could be compared favorably with the variability in such tests as bacterial or bacterial-spore counts in flour, within or between laboratories, we would probably be better satisfied with the present baking-test results.

The greater the experimental error in any test result, the less chance we have of differentiating between the subjects under test or between the imposed test treatments. Replication of tests leads to an average which approaches the actual value. When differences between the subjects under test or responses to certain treatments are of high magnitude, then the baking test is a safe basis of evaluation in any one laboratory or even between laboratories as shown recently in collaborative studies conducted by J. M. Doty of the Nebraska Section, in which unanimous agreement was reached on the evaluation of four wheat varieties. Collaborative tests conducted recently by L. E. Leatherock of the Pioneer Section Research Committee on different Kansas wheat varieties showed a high degree of agreement between laboratories. In these cases the differences in the flour characteristics were rather pronounced.

Wheat selections, milling operations, and flour evaluations often demand a differentiation between samples that show less difference than in the above-mentioned collaborative studies. To arrive at the proper evaluation of samples in such work the experimental error must be kept at a minimum. Mechanization of many of the test-bake operations, especially dough mixing, punching, and sheeting, should, from a logical standpoint, eliminate much of the personal factor. A complete automatic sheeting and molding device should find its place in the test but experienced operators maintain that the opportunity to feel the dough at certain stages of the operations is desirable for estimating the dough characteristics. A fermented dough passed through the sheeting rolls is generally considered to be in good condition to be judged by the sense of feel. In the absence of mechanical means that can be readily, economically, and reliably utilized for measuring the fermented dough characteristics which we must know, we cannot eliminate completely the personal equation from the test. When we have progressed in our development of test-bake equipment to the point of recommending one type of mixer, one type of sheeter, and one type of baking pan we will be in position to rewrite the standard procedure for the baking test and study seriously the possibilities of

standardization by means of collaborative studies which have not been attempted since Harrel (1929) found a definite lack of consistent results between laboratories. His findings were discouraging to the extent that it has since been deemed inadvisable to expose this important test to the possibility of such a variable record of collaborative results.

With equipment standardized and handling methods definitely prescribed we should establish through collaborative study the degree of concordance and variability of results that the grain, milling, and flour trade and research and control organizations can expect between well controlled laboratories. Such knowledge will enable them to know to what extent they can rely on the test-bake evaluations to carry on their inter-related operations, and they should be fully aware that sound interpretations cannot go beyond the accuracy of the test results as ordinarily obtained. In the rewriting of the A.A.C.C. baking-test method we will have to consider the procedure with reference to the specific objectives of the test and we will possibly want to establish the basic straight-dough test as an all-round safe procedure, including such items as shortening in the formula, high sugar level, and proofing the dough to constant height as suggested by Sandstedt and Blish (1939). W. L. Heald (1939) has recommended 3% shortening as desirable for the basic formula. Landis and Frey (1936) have given estimates of adequate sugar levels to assure fermentation. With such a basic procedure supplementary tests may be applied to give responses that have greater meaning in our test-bake interpretations.

At this time I wish to summarize the recent progress in development of test-bake equipment that is pertinent to the standardization program:

The Hobart-Swanson attachment mixer which was approved by the baking committee and which has been obsolete for some time has again been made available in a slightly modified form to facilitate better mixing of 100 g. and 200 g. of flour. This mixer is now available, with its own motive power, as the National-Swanson mixer, or the modified bowl can be supplied to give the improved smaller-dough mixing effect with the old-type Hobart-Swanson attachment. Unfortunately this development came too late for this year's committee to furnish any reports on its use.

Since the last convention 18 National sheeters have been purchased by laboratories and put into use. Two papers on the use of this equipment are presented on today's program. A summary of replies to a questionnaire sent to most of the users of the machine on points associated with handling doughs through the sheeter supplied the following information:

Thirteen of the sheeters are motor driven, five are hand driven. Twelve use the sheeter for punching; four do not. Ten remove the dough from the fermentation jar by hand; five use a spatula. Six use dusting flour; nine do not. The roll-sheeter setting for punching (average of nine) was  $8/32''$  and the range  $10/32''$  to  $6/32''$ . The roll setting for sheeting (average of fourteen) was  $6/32''$  and the range  $10/32''$  to  $4/32''$ . Eight mold the dough tight; six mold loose; one uses a special procedure. Fourteen favored recommendation of the National sheeter as approved equipment for the Tentative A.A.C.C. Baking Test; two were not definitely in favor; one was noncommittal.

There were no important suggestions for improvement in the machine. Favorable comments on the sheeter were: It is a definite work saver. It definitely tends to improve the grain and texture of the loaf. The price is not prohibitive. Unfavorable comments were: It possibly does not reduce significantly the individual's variability. In the case of soft wheat it possibly does not help and may hinder the differentiation between flours. General comments were: Loaf age marks are critical to the degree of mechanical treatment and this makes very careful standardization of procedure necessary. It does not solve the test-bake problems—it is only a step in the right direction. It should be developed with a molder. Molding by hand and machine are not significantly different; the molder is not essential.

No committee efforts have been given to oven development; however, attention should be called to such improvements as the compartment rotating hearth described by Finney and Barmore (1939) and the compartment reel-type oven developed by the National Manufacturing Co., Lincoln, Nebraska.

### Recommendations

That the National-Swanson dough mixer be studied as a replacement for the Hobart-Swanson attachment—this investigation to include a comparison of the double planetary two-pin bowl and the single planetary three-pin bowl mixers.

That the power-driven National sheeter or a sheeter of the same roll size and adjustments and approximate speed be tentatively approved for the A.A.C.C. bread-baking test.

That a baking pan of the following dimensions be studied as a single-pan replacement for the two types of pans now in use and as a pan size suited to the loaf volume range of from 550 cc. to 750 cc.: 75 mm.  $\times$  115 mm. top dimension, 60 mm.  $\times$  100 mm. bottom dimension, 60 mm. deep.

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